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During this contract period we have examined approximately 25 immunomodulating drugs for their ability to stimulate various components of the specific and nonspecific immune systems as well as their ability to enhance resistance to influenza, herpes-, arena-, bunya- and flaviviruses. All of these compounds were able to stimulate at least one component of the immune system and some were able to stimulate many. Although there was no correlation between stimulation of a particular component of the immune system and resistance to virus infection, in general, drugs that were most efficacious, appeared to stimulate a broader range of immune functions. Thus, we were able to rank immunomodulators based on their ability to stimulate a variety of immune functions and viral resistance. This ranking has enabled us to select several promising drugs as potential candidates for further evaluation and use in man. These drugs include: Poly I:C-LC, (poly riboinosinic:poly ribocytidylic acid complexed with carboxymethyl cellulose and poly-L-lysine); CL 246738, [3,6-bis(2-piperidinoethoxy)acridine trihydrochloride]; Ampligen, (a mismatched analog of poly riboinosinic:poly ribocytydylic acid); the pyrimidinone ABPP, {2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone}; the Riker-3M compound {1-(2-methyl propyl)-1H-imidazo[4,5-c]quinolin 4-amine} and a hybrid recombinant human interferon rhuIFNα-B/D).

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BIOLOGY OF IMMUNOMODULATORS

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SUMMARY YEAR 1

In the first year we compared the effects of Poly I:C-LC, CL 24673 and Ampligen on clearance and organ localization of radiolabelled sheep erythrocytes (SRBC); peritoneal, splenic and liver cell phagocytosis; antibody PFC responses; splenic and liver natural killer (NK) cytotoxicity; specific T cell cytotoxicity; enumeration of lymphoid cell subpopulations; macrophage antiviral activity and serum interferon levels. We have also examined the effect of these agents on resistance to herpes, Punta Toro and Banzi virus infections.

All three agents tested were capable of stimulating the blood clearance (reticuloendothelial) function of mice, although the timing of stimulation varied from one drug to other. Poly I:C-LC caused transient inhibition (day 2) followed by stimulation (day 7); CL 246738 caused stimulation between days 2 and 4 post treatment and Ampligen caused transient stimulation (day 2). Poly I:C-LC had the strongest effect on phagocytic cells and this effect persisted for at least 7 days in the peritoneum. CL 246738 had a transient effect observed only on day 2 post treatment while Ampligen had minimal effects on phagocytic activity. Poly I:C-LC had mild suppressive effect on PFC responses when given before antigen and some augmenting effect when given after antigen. Suppression appeared to be due to a dilution of the PFC resulting from expansion of splenic nucleated cell population. Similarly, augmentation also appeared to be due to an enlargement of spleens in treated animals. CL 246738 was without significant effect in this assay. Both Poly I:C-LC and Ampligen may cause a reduction in the proportion of T and B lymphocytes, although the time of this reduction may differ for the two drugs. CL 246738 is without effect on this parameter. Both CL 246738 and Ampligen potentiated macrophage extrinsic antiviral activity: cells from CL 246738 treated mice were about 100 times more effective than control macrophages and cells from Ampligen treated mice were about 10 times more effective. All three drugs enhanced the NK cytotoxicity and serum interferon levels, although Poly I:C-LC had most profound effect on these parameters.

All three drugs can afford some protection in the HSV-1 induced pneumonitis model when given prophylactically on D -2 or on the day of virus challenge. However, none of the drugs was effective in treating influenza pneumonitis. Poly I:C-LC and CL 246738 were also effective as prophylactic treatment in Punta Toro induced hepatitis. However, only Poly I:C-LC was effective in treating HSV-1 induced hepatitis. Poly I:C-LC given prophylactically was most effective in treating Banzi virus induced encephalitis (90-100% protection) whereas CL 246738 and Ampligen were relatively less effective (20-60% protection). None of the drugs were effective against HSV-1 induced encephalitis.

SUMMARY YEAR 2

During this year we compared the effects of a number of different pyrimidinones (ABPP, ACPP, AIPP, ABMP, ABMFPP and ACDFPP) on clearance and organ localization of radiolabelled sheep erythrocytes (SRBC); macrophage cytotoxicity; prostaglandin secretion and serum interferon levels. We have also examined the effect of these agents on resistance to herpes, Aichi and Banzi virus infections.

Five of these agents (ABPP, ACPP, AIPP, ABMFPP and ACDFPP) were examined for their effects on the reticuloendothelial system function and they were all capable of stimulating this function when tested two days after treatment. However these effects were not as pronounced four days after treatment. These pyrimidinones also caused a reduction in prostaglandin secretion by macrophages when given 2, 4 and 7 days before sampling. In addition, three of the pyrimidinones (ABPP, ACPP and ABMFPP) caused activation of macrophages to become cytotoxic whereas the other two (AIPP and ACDFPP) were without effect.
Of the five pyrimidinones tested for their effect on serum interferon levels, AIPP and ABMP produced a marginal increase, ABPP a moderate increase and ACPP and ABMFPP a large increase. In all cases the peak response was observed between days 1 and 2 post treatment.

The most beneficial effect of these pyrimidinones was observed in the Banzi virus encephalitis model. Four of the drugs (ACPP, AIPP, ABMFPP and ACDFPP increased resistance when given prophylactically and three (ABPP, ABMFPP and ACDFPP) when given on the day of challenge. In the herpesvirus encephalitis model, only ABPP affected the resistance and only when given prophylactically. Likewise, only ABMFPP had some effect in the influenza model and only when given on the day of challenge. None of the drugs were effective in the herpesvirus hepatitis model.

SUMMARY YEAR 3

During the third year of this contract we have focused our attention on PR 879-317A, AM-3, Isoprinosine the Riker-3M compound and two CIBA-GEIGY drugs, CGP 31,362 and rhuIFNα-B/D. CGP 31,362 is a small lipopeptide which has been reported to activate macrophages (CIBA-GEIGY, Biology Report, 1987) but not to induce interferon. RhuIFNα-B/D is a human α-interferon hybrid consisting of αB and αD subtypes. This interferon crosses species barriers and induces an "antiviral state" in murine cells. PR 879-317A is a pyridine derivative, AM-3 is a polyglucan, Isoprinosine is an inosine derivative and Riker-3M is a quinoline derivative. The parameters examined include: *in vivo* clearance and organ localization of radiolabeled sheep erythrocytes, changes in lymphocyte subpopulations, peritoneal and alveolar cell phagocytosis, and macrophage and natural killer cell cytotoxicity. In addition we have also examined the effects of these agents on resistance to infection in our viral models of pneumonitis, hepatitis and encephalitis.

In our functional assays AM-3 was able to augment the reticuloendothelial system and increase the number of phagocytic cells and phagocytic activity of individual cells. We were not, however, able to demonstrate significant *in vivo* activity in our murine models of pneumonitis, hepatitis and encephalitis.

Riker-3M was effective in stimulating the reticuloendothelial system, augmenting macrophage and natural killer cell cytotoxicity, and in increasing phagocytic activity. This drug was also effective in augmenting resistance to banzi virus encephalitis, when given by the oral route.

PR 879-317A was effective in enhancing reticuloendothelial system function; however, macrophage and natural killer cell cytotoxicity and peritoneal cell phagocytosis were not affected. Likewise, PR 879-317A was ineffective in our murine models of hepatitis, influenza and banzi encephalitis; however, some effect was observed in our murine model of HSV-1 induced pneumonitis.

CGP 31,362 augmented macrophage cytotoxicity and pulmonary phagocytic activity. This drug was able to induce resistance to HSV-1 pneumonitis but not HSV-1 induced hepatitis.

Enhanced reticuloendothelial system function, macrophage and natural killer cell cytotoxicity, and phagocytic activity was observed following the administration of rhuIFNα-B/D . This drug was also able to protect against parenteral challenge with banzi virus when given intravenously. When administered by the intracranial route, rhuIFNα-B/D was able to prolong the mean survival time of banzi virus infected animals but was not able to prevent death. However, mice receiving intracranial administration

of drug had markedly reduced banzi virus titers in their brains and spinal cords.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

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X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

PI - Signature DATE

Eugene P. Mayer

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I. PROBLEM UNDER INVESTIGATION

This study was designed to evaluate the multifaceted effects of selected immunoenhancing drugs on specific and nonspecific components of the immune system which are of importance in resistance to and recovery from viral infections. We have examined the effect of treatment schedule on various *in vitro* and *in vivo* immune parameters. The immune parameters examined included:

A. *In Vitro / Ex Vivo* Evaluation of Nonspecific Elements Affecting the Course of Viral Disease:

   1. Macrophage antiviral cytotoxicity

   2. Natural killer (NK) cell cytotoxicity

   3. Production of interferons (IF)

   4. Clearance of radiolabeled erythrocytes from blood and their localization in various organs

   5. Phagocytosis by peritoneal, splenic and liver macrophages

B. *In vitro / Ex Vivo* Evaluation of Specific Elements Affecting Resistance to and Recovery from Viral Diseases:

   1. Antibody responses to T-dependent antigens

   2. T cell cytotoxicity

   3. Alterations in T and B lymphocyte populations and subpopulations (*e.g.*, T helper or suppressor cells)

C. Evaluation of Host Resistance to and Recovery from Viral Infections:

II. BACKGROUND

Members of the military are exposed to a variety of viruses which often result in infections leading to serious illness or death. Although they can sometimes be protected by active immunization, this approach is not always practical due to difficulties in producing either attenuated or killed vaccines which are both safe and immunogenic. In addition, vaccines are of little value in the therapy of active viral infections. Therefore, alternative approaches have been explored. One approach has been the development of antiviral drugs. While these drugs have been effective, in some situations, their use has been hampered by their toxic side effects and limited range of activity.

Another approach to prevention and treatment of viral infections has been immunotherapy. Although immunotherapy with classical agents has had some success, it has also been plagued by toxicity problems. However, the recent development of chemically defined or synthetic immunostimulants with low toxicity and broad spectrum activity has made this approach more appealing. These immunostimulants have been used alone and in combination with vaccines in prophylaxis or with antiviral compounds in therapy.

While there are numerous reports of the efficacy of the newer generation immunostimulants, the experimental approaches utilizing these compounds have varied, thus, making an objective analysis of their comparative efficacy difficult. In addition, since the cellular components of the immune system that need to be stimulated will vary depending on the pathogenic features of the virus, it is essential that the mode of action of immunostimulating drugs be defined. Because the comparative efficacy and mode of action of many immunostimulants have not been fully explored their use has been mostly empirical. A more rational approach for the selection of appropriate drugs for use in prophylaxis or therapy requires 1) a comparison of the efficacy of various agents under the same experimental conditions and with the same panel of tests and 2) a better understanding of their modes of action.

Most immunostimulants possess a unique set of immunomodulating features and provide varying degrees of benefit to the infected host. The beneficial effects imparted by these immunostimulants will largely depend on the tissue site and degree of virus infection. For example, it may be desirable to have elevated levels of interferon in some tissue sites during a particular time of infection but not during others. This may be particularly relevant in some arenavirus infections (*e.g.*, lymphocytic choriomeningitis virus; LCMV) in which interferon can have detrimental effects (1, 2). Likewise, activated NK cells and macrophages may result in immunopathologic damage which can contribute to the disease process (3). Because of these complexities, the choice of immunomodulating agents, their dose, time

and frequency of administration require careful consideration of the immunopathologic features of infection. This is only possible if one is able to identify the spectrum of changes induced by a particular drug.

By virtue of their position at sites of initial infection and wide distribution in major organs of the body, macrophages and NK cells and their soluble mediators (*e.g.*, PG, IL, MAF and IF) are thought to be of prime importance in resistance to a number of intracellular pathogens. Thus, for many viral infections macrophage function has been shown to be an important factor in determining the course of the disease (4-7). For example, in herpesvirus infections both resistance to virus replication within macrophages (intrinsic resistance) and macrophage antiviral effects on other virus infected cells (extrinsic antiviral activity) may be significant determinants in host resistance. (8)

In addition to macrophages, another cell type which plays a significant role in primary resistance to virus infection is the NK cell (9-11). Unlike the cytotoxic T lymphocyte, this cell destroys virus infected cells without prior sensitization and thus quickly limits virus dissemination (11). A positive correlation between genetically determined resistance to virus lethality and the level of NK cell augmentation has been observed in both murine cytomegalovirus and herpes simplex virus infections (12,13).

A variety of soluble mediators may be released following the administration of various immunostimulants. Some of these mediators may have a negative effect on the immune system while others may have a positive effect. For example, prostaglandins may have a detrimental effect due to their negative feedback control on cellular functions (14-16). In contrast, interferon has a beneficial role in inhibition of virus replication as well as in the augmentation of cellular components of the immune system. While each type of interferon (*i.e.* alpha, beta and gamma) possess the ability to induce the antiviral state in cells, gamma interferon may be more important since it also regulates various immune functions (17-19).

There are a number of reports on the use of macrophage activators in the treatment of infectious diseases. Most notably, these compounds have been used prophylactically to enhance nonspecific resistance by direct activation of macrophages and NK cells or via the induction of soluble mediators. For example, inoculation of mice with *Escherichia coli* endotoxin, *Staphylococcus aureus*, BCG, or the lipoidal amine (CP-20,961) enhances resistance to influenza virus through the induction of interferon and/or the activation of macrophages and NK cells (20-23). Similar effects against herpesviruses, Newcastle disease, encephalomyocarditis, vesicular stomatitis, and Junin viruses were observed after treatment with various immunostimulants (24-30). Likewise, inoculation of mice with *P. acnes* induced protection against various hemoprotozoans (31-34).

In addition to their effects on macrophages and NK cells immunostimulating agents also affect elements of the specific immune response. Since both antibody and cell mediated immune responses are involved in resistance to and recovery from viral infections, immunostimulating drugs have been used in combination with whole, and subunit viral vaccines in an attempt to enhance their immunogenicity (35, 36). Use of immunostimulants may be particularly valuable in those situation in which cloned vaccines are available, since these antigens are poor immunogens.

Unfortunately, selection of appropriate immunostimulants to use with vaccines has been somewhat empirical. This is due to the variety of cellular targets on which immunostimulants can act, and the paucity of information concerning the their effects on these targets. For example, some immunostimulants, or the soluble mediators released in response to them, may selectively potentiate B cells, or suppressor or helper T cells which may influence the quantity of antibody produced following vaccination (37-39). In contrast, other immunostimulants may preferentially augment cytotoxic T cells which can have profound effects on recovery from viral disease but have little impact on resistance to viral infection.

In summary, immunoenhancing drugs can exert their effect by interacting with one or more of the cellular components of the immune system. These components are affected either directly, or indirectly through the action of soluble mediators. The ultimate outcome of such drug interactions will depend upon which of the various components is influenced. Therefore, the judicious use of immunoenhancing drugs, together with vaccines in prophylaxis or in the therapy of viral infections of military importance, requires a thorough understanding of their relative effects on the numerous components of the immune system.

While the prophylactic use of immunopotentiating substances has been widely studied, their therapeutic value has not been well documented. In addition, the comparative efficacy and mode of action of various immunostimulants against a variety of infectious agents (especially those of military significance) has not been adequately examined.

Our studies will provide the comparative data on a spectrum of immunological parameters for various immunoenhancing drugs. These data will provide a more scientific basis for the use of various immunoenhancing agents, either alone or in combination with vaccines or antivirals, in the effective treatment of viral diseases of importance to the military.

III. EXPERIMENTAL APPROACH

In this project each immunoenhancing drug was studied in two phases. During the first phase we examined the effects of selected drugs on a variety of components in the immune system. In the second phase we applied the knowledge gained from the initial phase to design experimental protocols to evaluate the clinical potential of these drugs. The studies were performed in animal models of human viral disease.

Phase I consisted of experiments designed to characterize the effects which selected immunostimulants exerted on the nonspecific or specific components of the immune system. Drugs were administered to C3H/HeN mice, intraperitoneally (i.p.), intravenously (iv.) or orally and appropriate cells or fluids obtained at selected intervals. The cells were examined *in vitro* for a variety of effector functions and their characteristic surface markers. The fluids were examined for the presence of soluble mediators. The effects of time of treatment were also assessed.

Phase II studies were designed to assess the effects of immunostimulants on resistance to and recovery from viral infection. Based on the immunological profiles from phase I and the pathogenesis of the viral agents under study, appropriate drugs were selected for either prophylaxis or therapy. Animals were examined for their ability to survive challenge with lethal doses of infectious agent. These experiments were performed using murine models of influenza virus herpesvirus, Punta Toro and Banzi virus infections. Lung, liver and brain infections were studied. The following animal models were employed.

Influenza Virus Pneumonitis: The virus used in these studies is a mouse adapted H3N2 strain of influenza A virus (Aichi). When 2-10 LD_{50} of this strain is administered intranasally into six to seven week old C3H/HeN mice, death, due to interstitial pneumonia, occurs in five to seven days. Virus is found only in the lungs and mice eventually die of pneumonia.

HSV-1 Pneumonitis: The virus used in these studies is a human isolate (VR3 strain) of type 1 herpes simplex virus (HSV-1) obtained from Dr. Andre Nahmias (Emory University, Atlanta, GA). Intranasal inoculation of three to five week old C3H/HeN mice with 2-10 LD_{50} of virus results in a fulminant pneumonitis and adrenalitis. Death occurs five to eight days following infection. A unique aspect of this model is that encephalitis does not occur.

HSV-1 Encephalitis: The virus used to induce encephalitis is a human isolate (MB strain) of type 1 herpes simplex virus obtained from Dr. Richard Whitley (Univ. Ala, Birmingham, AL). Footpad inoculation of four week old C3H/HeN mice results in virus replication in the sciatic

nerve, spinal cord and brain. Mice die of encephalitis six to eight days after inoculation. Immunoperoxidase staining for viral antigen has been used to confirm this mode of virus dissemination.

HSV-1 Hepatitis: The MB virus strain was used to induce liver disease. When four to five week old C3H/HeN mice are inoculated intravenously with 2-10 LD_{50} of virus, the primary organ of initial infection is the liver. Viremia and dissemination to a number of other organs follows liver infection and death results five to seven days post infection.

Banzi Virus Encephalitis: The seed virus used in these studies was obtained from Dr. C.J. Peters (USAMRIID, Fort Detrick, MD). Working stocks of virus are prepared from suckling mouse brains. When inoculated subcutaneously, this virus replicates in peripheral lymphoid tissue and is carried to the spleen. Viremia results 2-4 days post infection and the virus enters the brain. Encephalitis is observed 6-8 days post infection. Death ensues 8-10 days following the administration of as little as 10 p.f.u.

Punta Toro Hepatitis: The seed virus (Adames strain) used in our studies was initially prepared by Dr. D. Pifat (USAMRIID, Fort Detrick, MD). Working stocks of this virus are prepared by passage of cloned virus in Vero cells. Cloned virus was obtained from Dr. W. Sidwell (Univ. Utah) following enrichment and further characterization of the virus prepared by Pifat. Subcutaneous inoculation of 10^4 p.f.u. of our seed virus into 4 week old C3H/HeN mice results in hepatocellular necrosis and death 4-7 days post infection. A unique aspect of this virus infection is the tissue tropism which appears to be restricted to the liver and spleen even in the presence of high levels of circulating virus in the blood.

IV. RESULTS

Year 1

During the first year of this contract, we have focused our studies primarily on the comparative effects of Ampligen, CL 246738 and Poly I:C-LC on a number of immunological parameters, although, we have also begun investigations on other drugs. The parameters examined included: *in vivo* clearance and organ localization of radiolabelled sheep erythrocytes (SRBC); peritoneal, splenic and liver cell phagocytosis; antibody plaque-forming cell PFC) responses; splenic and liver natural killer (NK) cytotoxicity; specific T cell cytotoxicity; enumeration of lymphoid cell subpopulations; macrophage antiviral activity and serum interferon levels. We have also examined the effect of these agents on resistance to herpes, Punta Toro and Banzi virus infections.

In Vivo Clearance and Organ Localization of Erythrocytes

Tables 1-4 contain data on the effect of Poly I:C-LC, Ampligen and CL 246738 on clearance rate of SRBC from circulation and their localization in liver, spleen and lung. The clearance rates are presented as T/2 and K-values. An increase in K-value reflects an increase in the rate of clearance and consequently a decrease in the half-life (T/2) of SRBC in circulation. Also listed in the tables are alpha values which represent clearance rates normalized for mouse body, spleen and liver weights. Thus, increased alpha values also represent increased clearance rates. Organ localization is presented as number of SRBC per mg wet tissue.

Two days after iv. administration of Poly I:C-LC there was a reduction in the clearance rate of SRBC which was not due to alterations in the body or organ weights (Table 1). This reduction was apparently due to reduced localization in liver which is the major organ for clearance of particulate material from circulation. The decreased clearance rate represents a real increase in the SRBC half-life, since essentially all of the labelled cells were shown to be free (*i.e.*, not internalized) in the circulation. Also, the increased localization of SRBC in the spleen and the lung was not due to increased plasma volume and represents SRBC that have been phagocytosed. Furthermore, the alterations listed above were not due to the action of lysine-carboxymethylcellulose component of Poly I:C-LC (see Table 1, quarterly report #2).

The reduction in clearance rate observed on day 2 following Poly I:C-LC injection was not seen 4 days after treatment, and at this time, there was an increased localization in liver. By 7 days after treatment there was an increased clearance rate which could be accounted for by increased liver weight and increased hepatic localization. By 14 days after treatment no differences were observed between control and treated animals. Thus, Poly I:C-LC

causes a transient impairment (day 2) of the reticuloendothelial system followed by a slight enhancement (day 7). These effects were apparently due to the effect of the drug on liver function.

In contrast to Poly I:C-LC, both CL 246738, given orally, and Ampligen, given iv., increased blood clearance rate 2 days after treatment which appeared to be due to increased liver localization (Tables 2 & 3). The effect of CL 246738 was still evident on day 4 after treatment (Table 2) while no effect of Ampligen was seen at this time (Table 3). By 7 days after treatment neither CL 246738 nor Ampligen had any effect. The effect of CL 246738 given iv., even approaching toxic doses, was negligible (Table 4). Thus, Ampligen and CL 246738 have similar effects (stimulation) on clearance rate whereas Poly I:C-LC has an opposite effect (inhibition). In all cases these effects are transient.

In summary, all three agents tested are capable of stimulating the blood clearance function (reticuloendothelial function) of mice, although the timing of stimulation varies from one drug to other. Poly I:C-LC causes transient inhibition (day 2) followed by stimulation (day 7), CL 246738 causes stimulation between days 2 and 4 posttreatment and Ampligen causes transient stimulation (day 2).

Peritoneal, Splenic and Liver Cell Phagocytosis

Peritoneal, splenic and liver cell phagocytic activity was assessed as described in our third Quarterly Report. Data from representative experiments are shown in Figures 1-4. Poly I:C-LC given two days prior to test increased the phagocytic activity of peritoneal cells as evidenced by the increased fluorescence intensity (*i.e.*, greater number of internalized fluorescent bacteria) in the drug treated group (Figure 1). Furthermore, drug treatment also increased the proportion of phagocytic cells in the peritoneal population (15% vs 37% for highly phagocytic cells; channels 128-255). These effects were still evident on day 4 after treatment (Figure 2), began to wane on day 7 (Figure 3) and were near normal by day 14 (Figure 4). Results on the effects of Poly I:C-LC on splenic cells were somewhat variable: in one experiment there was evidence for an increase in the number of phagocytic cells (8% vs 16% for highly phagocytic cells) without increased fluorescence intensity whereas in another experiment there was no alteration. In a preliminary experiment, liver phagocytic cells from Poly I:C-LC treated animals exhibited an inhibition of activity and a decrease in the number of phagocytic cells (31% vs 11% for highly phagocytic cells), which is consistent with the results obtained in the clearance experiments described above.

Treatment with CL 246738 also increased the activity and number of phagocytic peritoneal cells (26% vs 39% for highly phagocytic cells) 2 days after treatment. However, by 4 days after treatment

no significant differences were observed between controls and treated
groups. Similarly, CL 246738 had little effect on splenic or liver phagocytic cells when administered 2 or 4 days prior to assay.

Treatment with Ampligen did not increase the number of phagocytic cells in the peritoneal population 2 days after treatment, although in one experiment there was some indication of increased phagocytic activity. No effect was observed 4 days after treatment. Similarly, Ampligen had no apparent effect on the number or activity of splenic or liver phagocytic cells at any time tested.

In summary, Poly I:C-LC had the strongest effect on phagocytic cells and this effect persisted for at least 7 days in the peritoneum. CL 246738 had a transient effect observed only on day 2 post treatment while Ampligen had minimal effects on phagocytic activity.

Antibody Plaque-Forming Cell Responses

The effect of Poly I:C-LC on antibody PFC was somewhat variable. When given before antigen, some suppression of plaque forming cells per million splenocytes was observed (Table 5). However, this reduction was apparent for only IgG PFC when the total number of PFC was calculated. This discrepancy apparently due to expansion of the splenic mononuclear cell population after drug treatment. When administered after antigen, Poly I:C-LC either did not alter the plaque forming cell response or, sometimes, it caused a slight enhancement, as exemplified by the IgM PFC per spleen (Table 5).

Generally, CL 246738 did not have any effect on the plaque forming cell response whether given before or after antigen (Table 6). The significance of reduction in the IgG PFC per 10^6 will have to be reevaluated. However, Ampligen showed some similarities with Poly I:C-LC in that it suppressed IgG PFC per 10^6 when given before antigen and enhanced the IgM PFC per spleen when given after antigen (Table 7).

In summary Poly I:C-LC had mild suppressive effect on PFC responses when given before antigen and some augmenting effect when given after antigen. Suppression appeared to be due to a dilution of the PFC resulting from expansion of splenic nucleated cell population. Similarly augmentation also appeared to be due to an enlargement of spleens in treated animals. CL 246738 was without significant effect in this assay.

Splenic and Liver Natural Killer Cells

The effects of Poly I:C-LC, CL 246738 and Ampligen on splenic and liver NK cytotoxicity was assessed as described in our second and third Quarterly Reports and the data is presented in Tables (8-10).

Poly I:C-LC, given 2 days prior to assay, was able to augment splenic
NK activity and this augmentation persisted for at least 4 days (Table 8). However, on day 7 or 14, no significant augmentation in spleen NK cell activity was observed. Although we have not as yet examined the effects of Poly I:C-LC, given 2 and 4 days prior to assay, on liver NK cell activity, the data in Table 8 demonstrate that Poly I:C-LC may be able to augment liver NK activity for at least 14 days after treatment. These observations will be confirmed shortly.

CL 246738 was similar to Poly I:C-LC in being able to augment splenic NK activity day 2 and day 4 after treatment (Tables 9). However, this drug was not able to augment liver NK activity using the same treatment schedules. We are currently repeating these observations and investigating whether CL 246738 will augment splenic and liver NK cell activity after 7 or 14 days.

Ampligen like Poly I:C-LC and CL 246738 was able to augment splenic NK cell activity 2 days after treatment, however, unlike Poly I:C-LC and CL 246738 no significant augmentation was observed 4 days after treatment (Table 10). Also, like CL 246738, Ampligen had little effect on liver NK activity (Table 10).

In summary, all three drugs, Poly I:C-LC, CL 246738 and Ampligen were able to augment splenic NK activity, but the effect was transient, lasting less than 7 days for Poly I:C-LC and 4 days for CL 246738 and Ampligen. Only Poly I:C-LC augmented liver NK cytotoxicity.

Specific T Cell Cytotoxicity

We have recently begun studies on the effects of drugs on specific T cell cytotoxicity. The design of these experiments is as follows. Animals are treated with drug, sacrificed various times thereafter and their spleen cells are incubated *in vitro* with mitomycin-C treated allogeneic cells for 4-5 days. Specific T cell cytotoxicity of sensitized splenocytes is then measured using ^{51}Cr-labeled target cells with the same H-2 haplotype as the stimulator cells.

Our initial studies indicate that treatment with Poly I:C-LC, 2 days prior to *in vitro* sensitization, resulted in a significant decrease in specific T cell cytotoxicity whereas treatment with CL 246738 had no significant effect (Table 11). We are currently repeating these studies and expanding these interesting observations.

Enumeration of Lymphoid Cell Subpopulations

Splenic lymphocyte subpopulations were enumerated by treating cells from control and drug treated animals with fluorescenated antibodies and determining the number of fluorescent cells on the flow cytometer. The antibodies used included: anti-IgG, for B cells; monoclonal anti-Thy-1, for T cells; monoclonal anti-Ly-1 and anti-Ly-2 for the
Ly1+2+, Ly1+2- and Ly 1-2+ T cell subpopulations. The data obtained to date are presented in Table 12. As can be seen in this table, treatment with Poly I:C-LC on day -2, -4 or -14 had no effect on the splenic lymphocyte subpopulations. Treatment with Poly I:C-LC on day -7 may decrease the number of B cells, T cells and T cell subpopulations but this observation remains to be confirmed. CL 246738 treatment did not appear to have an effect on the lymphocyte subpopulations. In contrast, treatment with Ampligen may decrease the proportion of B cells, T cells and T cell subpopulations but a definite conclusion must await our repeating these experiments.

In summary, Poly I:C-LC and Ampligen may both cause a reduction in the proportion of T and B lymphocytes, although the time of this reduction may differ for the two drugs. CL 246738 is without effect on this parameter.

Macrophage Antiviral Activity

Macrophage extrinsic antiviral activity was assessed by culturing peritoneal macrophages from control or drug treated animals with HSV-1 infected Vero cells for 3 days and measuring the reduction in virus titers. As can be seen from Table 13, macrophages from control animals were able to reduce the virus titers by 1.76 logs while macrophages from CL 246738 and Ampligen treated mice reduced titers by 3.61 and 2.88, respectively. Thus, both CL 246738 and Ampligen potentiated macrophage extrinsic antiviral activity: cells from CL 246738 treated mice were about 100 times more effective than control macrophages and cells from Ampligen treated mice were about 10 times more effective.

Interferon Levels

Serum interferon levels following treatment with Poly I:C-LC, CL 246738 or Ampligen were examined on days, 1, 2, 3, 4 and 8 following drug treatment using a VSV plaque reduction assay. As can be seen in Figure 5, the response to all three drugs peaks 1 day after treatment and is significantly reduced by day 3. Interferon concentrations reach background levels by day 8. Moreover, Poly I:C-LC given iv. was a more effective interferon inducer than Ampligen given by the same route or CL 246738 given orally.

Resistance to Herpes, Punta Toro and Banzi Virus Infections

The ability of Poly I:C-LC, CL 246738 and Ampligen to enhance antiviral resistance was examined in murine models of pneumonitis, hepatitis and encephalitis. The results of these experiments are presented in Figures 6-14.

Pneumonitis Models

Two viruses which induce murine pneumonitis were employed, influenza (Aichi strain) and HSV-1. In these models 10 LD_{50} of virus was administered intranasally and mortality monitored for 21-30 days. As can be seen in Figure 6, 100 μg of Poly I:C-LC, administered on the day of or 2 days prior to challenge with influenza virus, was unable to protect the mice. In contrast, 70-80% protection was obtained in the HSV-1 pneumonitis model when Poly I:C-LC was given on the same schedule. However, Poly I:C-LC was ineffective if given therapeutically 1 day after virus challenge.

Like Poly I:C-LC , CL 246738 and Ampligen were unable to protect mice challenged with influenza virus (Figures 7 and 8). However, CL 246738 treatment gave some (30%) protection when given on the day of virus challenge and moderate (50%) protection when given prophylactically on D -2 (Figure 7). Ampligen was equally effective as CL 246738 in protecting when given prophylactically on D -2 and perhaps slightly more effective when given on the day of virus challenge (Figure 8).

In summary, all three drugs can afford some protection in the HSV-1 induced pneumonitis model when given prophylactically on D -2 or on the day of virus challenge. However, none of the drugs was effective in treating influenza pneumonitis.

Hepatitis Models

Two viruses which induce murine hepatitis were employed in our studies, HSV-1 (given iv.) and Punta Toro (given ip.). In these models 10 LD_{50} of virus was administered by the route indicated above and mortality monitored for 21-30 days. Poly I:C-LC was effective (70% protection) in treating HSV-1 induced hepatitis when given prophylactically on D -2 but it was apparently ineffective when given on the day of virus challenge (Figure 9). Similarly, Poly I:C-LC was able to protect (100 %) mice challenged with Punta Toro virus when given prophylactically.

Unlike Poly I:C-LC, neither CL 246738 nor Ampligen were able to protect against HSV-1 induced hepatitis even when given prophylactically (Figures 10 & 11). However, CL 246738 was

completely protective in the Punta Toro virus model. Ampligen has not yet been tested in this model.

In summary, both Poly I:C-LC and CL 246738 can be used to treat Punta Toro induced murine hepatitis using a prophylactic protocol. However, only Poly I:C-LC is effective in treating HSV-1 induced hepatitis.

Encephalitis Models

Two models of encephalitis were employed, one which uses HSV-1 (given via the foot pad) and the other which uses Banzi virus (given ip.).
Poly I:C-LC was able to give good protection (90%-100%) only in the Banzi virus model and only when given prophylactically or on the day of virus challenge (Figure 12). Only slight protection (20%) was observed when the drug was given therapeutically 1 day after challenge. CL 246738 also protected in the Banzi virus model when given prophylactically but the protection was not as good (20 % when given on D -0 and 60 % when given on D -2) as was seen with Poly I:C-LC (Figure 13). No protection was observed when CL 246738 was given therapeutically. In the Banzivirus model, the effects of Ampligen were similar to CL 246738. (Figure 14)

In summary, Poly I:C-LC given prophylactically was the best in treating Banivirus induced encephalitis (90-100% protection) while CL 246738 and Ampligen were less effective (20-60% protection). None of the drugs was effective against HSV-1 induced encephalitis.

Year 2

During the second year of this contract, we have focused our studies primarily on the comparative effects of various pyrimidinones on a number of immunological parameters, although, we have also begun investigations on other drugs. The parameters examined included: *in vivo* clearance and organ localization of radiolabelled sheep erythrocytes (SRBC); peritoneal and splenic cell phagocytosis and activation of cytotoxic macrophages. We have also examined the effect of these agents on resistance to viral models of pneumonitis, hepatitis and encephalitis.

In Vivo Clearance and Organ Localization of Erythrocytes

Tables 14-15 contain data on the effect of the various pyrimidinones on clearance rate of SRBC from circulation and their localization in liver, spleen and lung. The clearance rates are presented as T/2 and K-values. An increase in K-value reflects an increase in the rate of clearance and consequently a decrease in the half-life ($T_{1/2}$) of SRBC in circulation. Also listed in the tables are alpha values which represent clearance rates normalized for mouse body, spleen and liver weights. Thus, increased alpha values also represent increased clearance rates. Organ localization is presented as number of SRBC per mg wet tissue.

Two days after intraperitoneal (ip) administration of all pyrimidinones tested caused an increase in the clearance rate of SRBC which did not appear to be due to alterations in the body or organ weights as indicated by the increase in alpha values. These effects, with the exception of ACPP, were statistically significant (Table 14). This increase was apparently due to increased localization in liver which is the major organ for clearance of particulate material from circulation. Since all pyrimidinones were administered in carboxymethylcellulose (CMC), it was also necessary to compare the CMC treated group with a saline control group. Such a comparison revealed that CMC itself stimulated the reticuloendothelial functions. Consequently, the effect of drug-carrier mixture was more pronounced when compared with the saline control.

The effects of pyrimidinones were less pronounced when drugs were administered four days before assay as compared to the CMC group. However, these effects were still mostly significant when compared with the saline control (Table 15).

Macrophage Cytotoxicity

Macrophage cytotoxicity was tested by incubating peritoneal adherent cells with virally transformed EL-4 cells for 48 hours and measuring

the incorporation of ^{3}H-thymidine by the target cells. In these experiments macrophages were harvested four days after ip injection of drugs. Results summarized in Table 16 indicate that treatment with ABPP, ACPP and ABMFPP caused activation of macrophages to become cytotoxic when compared with macrophages from CMC-treated controls. In this assay, CMC treatment was without effect (compared with the saline control). The cytotoxicity was significant at all effector to target ratios ranging from 40:1 to 10:1. In contrast, AIPP and ACDFPP had no significant effect.

Prostaglandin Secretion by Macrophage

Mice were treated with CMC or pyrimidinones in CMC and peritoneal cells were harvested 2, 4, 7 or 14 days later. Adherent cells were cultured for 20 hours and prostaglandin E-2 levels in supernatants were measured by radioimmunoassay. The results have been summarized in Tables 17-21. It is clear that all pyrimidinones caused reduction in prostaglandin secretion by macrophages when given 2, 4 or 7 days before sampling. When macrophages were harvested 14 days after treatment, this effect was variable.

Interferon Levels

Serum interferon levels following treatment with the various pyrimidinones were examined on days, 1, 2, 3, 4 and 7 following drug administration using a VSV plaque reduction assay. These data are summarized in Figures 15-19. Although all pyrimidinones tested caused a noticeable elevation in serum interferon level, the magnitude of the response varied with each drugs. A marginal increase, which was only slightly above the CMC control, was observed with AIPP and ABMP (Figures 15 and 16). ABPP on the other hand produced a moderate increase (Figure 17) and ACPP and ABMFPP had a more dramatic effects (Figures 18 and 19). In all case the peak elevation was observed between 1 and 2 days post treatment and was back to background levels by days 3 and 7.

Resistance to Herpes, Influenza and Banzi Virus Infections

The ability of different pyrimidinones to enhance antiviral resistance was examined in murine models of pneumonitis, hepatitis and encephalitis. The results of these experiments are presented below.

Pneumonitis Models

Influenza virus (Aichi strain) was used to induce pneumonitis. In this model 10 LD_{50} of virus was administered intranasally and mortality monitored for 21 days. Data from these experiments are summarized in Figures 20-37. ABMFPP, when given on the day of

challenge, had a slight, although statistically significant, effect on the mean survival time of infected animals. This treatment also afforded some protection against the infection as 2/10 mice survived until the termination of the experiment (day 21 post-infection) (Figure 33). However, ABMFPP was without effect when given 2 days before, or one day after virus challenge (Figures 32 and 34 respectively). All other pyrimidinones, whether given two days before, on the day of, or one day after challenge, were ineffective.

Hepatitis Models

Herpesvirus (MB-strain) was used to induce hepatitis. In this model 10 LD_{50} of virus was administered by the iv. route and morbidity and mortality monitored for 21 days. None of the pyrimidinones offered any protection in this model whether given 2 days before, on the day of, or one day after virus infection (Figures 38-55).

Encephalitis Models

Two models of encephalitis were employed, one which uses HSV-1 (given via the foot pad) and the other which uses Banzi virus (given ip.). Data from the herpesvirus experiments are summarized in Figures 56-73. ABPP, when given 2 days before challenge, had a slight, although statistically significant, effect on the mean survival time of infected animals. ABPP also afforded some protection against the infection as 2/10 mice survived until the termination of the experiment (day 21 post-infection) (Figure 56). However, this drug was without effect when given on the day of, or one day after virus challenge (Figures 57 and 58). All other pyrimidinones, whether given two days before, on the day of, or one day after challenge, were ineffective.

The effects of different pyrimidinones on Banzi virus induced encephalitis are summarized in Figures 74-91. When given 2 days before infection, ACPP, AIPP, ABMFPP and ACDFPP had some beneficial effects in prolonging the mean survival time (Figures 77, 80, 86, 89). Three of these also afforded some protection: 1/10 with AIPP (Figure 80) and 2/10 with ABMFPP or ACDFPP (Figures 86 and 89). When given on the day of challenge, ABPP, ABMFPP and ACDFPP prolonged the mean survival time, although none of these drugs afforded any protection (Figures 75, 87, 90). None of the drugs conferred resistance to this virus when given one day after the infection (Figures 76, 79, 82, 85, 88 and 91).

Year 3

During the third year of this contract we have focused our attention on PR 879-317A, AM-3, Isoprinosine the Riker-3M compound and two CIBA-GEIGY drugs, CGP 31,362 and rhuIFNα-B/D. CGP 31,362 is a small lipopeptide which has been reported to activate macrophages (CIBA-GEIGY, Biology Report, 1987) but not to induce interferon. RhuIFNα-B/D is a human α-interferon hybrid consisting of αB and αD subtypes. This interferon crosses species barriers and induces an "antiviral state" in murine cells. PR 879-317A is a pyridine derivative, AM-3 is a polyglucan, Isoprinosine is an inosine derivative and Riker-3M is a quinoline derivative. The parameters examined include: *in vivo* clearance and organ localization of radiolabeled sheep erythrocytes, changes in lymphocyte subpopulations, peritoneal and alveolar cell phagocytosis, and macrophage and natural killer cell cytotoxicity. In addition, we have also examined the effects of these agents on resistance to infection in our viral models of pneumonitis, hepatitis and encephalitis.

In Vivo Clearance and Organ Localization of Erythrocytes

Tables 22-27 contain data on the effect of AM-3, Riker-3M, PR 879-317A and isoprinosine on clearance rate of SRBC from circulation and their localization in liver, spleen and lung. The clearance rates are presented as T/2 and K-values. An increase in K-value reflects an increase in the rate of clearance and consequently a decrease in the half-life (T/2) of SRBC in circulation. Also listed in the tables are alpha values which represent clearance rates normalized for mouse body, spleen and liver weights. Thus, increased alpha values also represent increased clearance rates. Organ localization is presented as number of SRBC per mg wet tissue.

As indicated in Table 22 when AM-3 was administered intraperitoneally (400 mg/kg) two days prior to assay, a significant enhancement of SRBC clearance from the circulation was observed. This effect was not, however observed when drug was administered four days prior to assay. Similar results were observed when Riker 3M (10 mg/kg) or PR 879-317A (10 mg/kg) were administered intraperitoneally or intravenously (Tables 23 and 24, respectively). However, when PR 879-317A was given at 50 mg/kg, some activity was still apparent four days after treatment (Table 25). It should be noted that the Riker-3M drug was effective when given orally or intraperitoneally (Figure 92). Thus, these drugs were effective to some degree in enhancing the function of the reticuloendothelial system. In contrast to the above, isoprinosine (500 mg/kg) was not effective in stimulating the reticuloendothelial system when administered 2, 4 or 7 days prior to assay (Table 26).

As illustrated in Table 27, mice receiving exogenous interferon (rhuIFNα-B/D) at 1 x 10^6 units, 24 hours prior to the intravenous injection of chromium-labeled SRBC, cleared these cells from their circulation more rapidly than control mice. As expected, SRBC accumulated in the liver of treated mice indicating enhanced RES function following interferon treatment. It should be noted that both the i.v. and i.p. routes of administration were effective in stimulating the reticuloendothelial system.

Macrophage Cytotoxicity.

Macrophage cytotoxicity was tested by incubating peritoneal adherent cells with virally transformed EL-4 cells for 48 hours and measuring the incorporation of ^{3}H-thymidine by the target cells. In these experiments macrophages were harvested four days after ip injection of drugs. Results summarized in Table 28 indicate that only minor changes in macrophage cytotoxicity were observed at either two or four days following the oral administration of 10 mg/kg of Riker 3M (Table 28), however, these changes were not significant. Likewise, no augmentation of macrophage cytotoxicity was observed following intraperitoneal administration of drug (data not shown). Similar results were obtained with PR 879-317A (Table 29).

As shown in Table 30, CGP 31,362 at 100 μg/kg gave significant cytotoxic activity at a target to effector cell ratio of 40:1 and 20:1. The lack of effective cytotoxic responses at a 10:1 ration may be due to a decrease in the number of macrophages present in inflammatory infiltrates. Such dilution effects could result from the neutrophilia induced following CGP 31,362 administration (Biology Report, CIBA-GEIGY, 1987).

Table 31 illustrates the cytotoxic activity of peritoneal macrophages 1 day following the intraperitoneal injection of 1 x 10^6 units of rhuIFNα-B/D. As illustrated, interferon was as effective as poly I:C-LC in enhancing the cytotoxic potential of peritoneal macrophages.

Splenic Natural Killer Cells

The effects of Riker-3M, PR 879-317A, isoprinosine and recombinant α-interferon on splenic NK cytotoxicity was assessed as described in our previous reports and the data is presented in Tables (32-34).

Riker 3M enhanced splenic NK cytotoxicity when administered orally two days prior to assay (Table 32). This effect was significant at each of the three effector to target cell ratios examined. Intraperitoneal administration of drug was also effective in enhancing splenic NK cytotoxicity. Note that the vehicle, lactic

acid, had no effect. In contrast PR 879-317A and isoprinosine were ineffective in augmenting splenic natural killer cell activity when administered two or four days prior to assay (Table 33).

Table 34 illustrates the NK activity in splenocytes from mice receiving 1 x 10^6 units of interferon (i.v.or i.p.), 1 day prior to assay. Note that the i.p. route was superior to the i.v. route of administration and that significant enhancement of NK activity was observed at each of the three effector to target cell ratios.

Peritoneal and Alveolar Cell Phagocytosis

Riker 3M (10 mg/kg) was effective in increasing the number of phagocytic cells in peritoneal exudates two days following oral administration (Figure 93). No augmentation of PEC phagocytosis was observed following intraperitoneal administration of Riker 3M (Figure 94).

As indicated in Figures 95 and 96 intraperitoneal administration of AM-3 (400 mg/kg), either two or four days prior to harvesting peritoneal exudate cells resulted in a significant increase in the number of phagocytic cells and phagocytic activity of individual cells. In contrast, PR 879-317A was ineffective in this assay (Figures 97 and 98).

Since previous reports (CIBA-GEIGY) had indicated that CGP 31,362 was effective in enhancing pulmonary immunity, the phagocytic activity of alveolar macrophages was examined two days after intranasal instillation of 100 μg/kg of CGP 31,362. As illustrated in Figure 99, the total number of phagocytic cells increased from 34 to 52% following drug treatment but the phagocytic activity of individual cells did not change.

Figure 100 illustrates the phagocytic activity of peritoneal macrophages 24 hours after intraperitoneal injection of 1 x 10^6 units of rhuIFNα-B/D. Note that the total number of phagocytic cells recovered from the peritoneum did not change significantly. Nonetheless, the phagocytic activity of peritoneal macrophages from interferon treated mice was significantly enhanced. In one experiment (data not shown) we compared i.v. to i.p. routes of inoculation. There was no effect observed following i.v. inoculation, indicating that interferon exerts its effect on local macrophages. This is consistent with the effect of interferon on activation of natural killer cells (see above).

Enumeration of Lymphoid Cell Subpopulations

Splenic lymphocyte subpopulations were enumerated by treating cells from control and drug treated animals with fluorescenated antibodies and determining the number of fluorescent cells by flow cytometry. The antibodies used were anti-IgG, for B cells and

monoclonal anti-Thy-1, for T cells. Table 35 illustrates the cell subpopulations observed in spleens from mice which received an intravenous injection of 1 x 10^6 units of rhuIFNα-B/D. There were no significant changes in the total number of cells present in the spleen but the percentage of B cells was slightly reduced following i.v. inoculation as was the percentage of T cells following i.p. inoculation. Note however, that the absolute numbers of B or T cells did not change significantly following interferon administration.

Resistance to Virus Infections-(Pneumonitis/Hepatitis/Encephalitis)

Riker 3M was effective in augmenting resistance to Banzi encephalitis when administered orally or intraperitoneally on the day of virus challenge (Figure 101). However, protection was not observed when this drug was administered by gavage on either day one or two post-infection (Figure 102).

In spite of the fact that AM-3 was effective in some of our *in vitro* functional assays, we were unable to show any enhancement in resistance in our murine models of pneumonitis, hepatitis or encephalitis (Figures 103-105, respectively). Note that in the banzi virus model, this drug may have exacerbated the disease (Figure 105).

PR 879-317A significantly enhanced resistance to HSV-1 induced pneumonitis when administered intraperitoneally (10 mg/kg) one day after virus challenge (Figure 106). In contrast, drug administered by the intravenous route was ineffective in this model. Note also that this compound was ineffective in murine models of HSV-1 hepatitis, influenza and Banzi encephalitis (Figures 107-109, respectively).

When administered by the intranasal route three days prior to, on the day of, and one day following intranasal instillation of HSV-1, CGP 31,362 therapy resulted in significant protection (Figures 110 & 111). Note, however, that neither the intraperitoneal nor the intravenous routes of delivery were effective in therapy of HSV-1 pneumonitis. In contrast to pneumonitis, CGP 31,362 was not able to enhance resistance to HSV-1 hepatitis regardless of the route of administration (data not shown).

As demonstrated in Figure 112, most (75%) of the mice receiving a single intravenous inoculation of interferon (1 x 10^6 units) on the day of banzi virus challenge did not develop disease symptoms and survived infection. Mice receiving 1 x 10^6 units one day after infection lived significantly longer than controls but eventually developed encephalitis and died. No protection was observed when mice were treated with interferon at later times (day 2 or 3) following infection.

When administered intracranially via cerebroventricular injection, rhuIFNα-B/D (1 x 10^6 units) was able to prolong the mean survival time of Banzi infected animals but was not able to prevent them from dying (Figure 113). Note that interferon administered one day after infection appeared to give the best results; however, day 0 and day 2 post-infection schedules were also effective. Moreover, the amount of infectious virus isolated from the brain and spinal cords of rhuIFNα-B/D treated mice (day 0 and day 1) was significantly lower than that observed in untreated controls (Table 36).

During this year we also examined the prophylactic activity of CL 246738 in mice treated at 2, 4, 6, 8, or 10 days prior to infection with Banzi virus. In this experiment, a single dose of drug (200 mg/kg) was administered by gavage prior to infection with 1 LD_{80} of Banzi virus. Figure 114 illustrates the protection afforded mice receiving prophylactic therapy. As illustrated, significant protection was afforded when drug was administered up to six days prior to virus infection. Protection dropped to 50% at eight days and was back to control levels (10% survival) when drug was administered ten days prior to virus infection.

V. CONCLUSIONS

The generated in the first year of this study has resulted in the following conclusions:

1. Poly I:C-LC alters the ability of mice to clear particulate material from circulation, uptake by liver and spleen in a variable manner: it is inhibitory on day 2 post treatment but stimulatory on day 7.

2. Poly I:C-LC stimulates the phagocytic activity of peritoneal macrophages

3. Poly I:C-LC appears inhibitory for antibody PFC response when given before antigen but is stimulatory when given after.

4. Poly I:C-LC enhances serum interferon levels

5. Poly I:C-LC augments NK cell cytotoxicity

6. Poly I:C-LC, given prophylactically, is protective in HSV, but not influenza, induced pneumonitis, HSV and Punta Toro virus hepatitis and Banzi virus encephalitis.

7. CL 246738 transiently stimulates *in vivo* clearance and *in vitro* phagocytic function of peritoneal cells.

8. CL 246738 enhances serum interferon levels.

9. CL 246738 augments NK cell cytotoxicity.

10. CL 246738 given prophylactically, is protective in HSV, but not influenza, induced pneumonitis, Punta Toro virus hepatitis and Banzi virus encephalitis.

11. Ampligen transiently stimulates *in vivo* clearance.

12. Ampligen may affect antibody PFC response in a manner similar to Poly I:C-LC.

13. Ampligen enhances serum interferon levels.

14. Ampligen augments NK cell cytotoxicity and its effect may be longer lasting than that of CL 246738.

15. Ampligen given prophylactically, is protective in HSV, but not influenza virus induced pneumonitis and Banzi virus encephalitis.

The generated in the second year of this study has resulted in the following conclusions:

1. All pyrimidinones had some RES stimulatory effect.

2. Three of the pyrimidinones cause activation of macrophages to become cytotoxic.

3. All pyrimidinones cause a reduction in prostaglandin secretion.

4. All pyrimidinones cause some increase in serum interferon levels.

5. Only ABMFPP had some protective effect against influenza.

6. None of the pyrimidinones conferred resistance against hepatitis.

7. Only ABPP had slight protective effect against herpesvirus induced encephalitis by several of the pyrimidinones increased resistance against Banzi virus induced encephalitis.

The data generated in the third year of this study has resulted in the following conclusions:

1. AM-3 was effective in augmenting the reticuloendothelial system when administered two days but not four days prior to examination.

2. When AM-3 was administered either two or four days prior to harvesting peritoneal exudate cells, a significant increase in the number of phagocytic cells and their phagocytic activity was observed.

3. Although AM-3 was effective in some of our *in vitro* functional assays, it was ineffective in our murine models of pneumonitis, hepatitis or encephalitis.

4. Riker-3M administered by either the oral or intraperitoneal routes, was effective in stimulating the reticuloendothelial system when administered two days prior to assay.

5. No augmentation of macrophage cytotoxicity was observed following intraperitoneal administration of Riker-3M.

6. Riker-3M enhanced splenic NK cytotoxicity when administered orally two days prior to assay.

7. Riker-3M was effective in increasing the number of peritoneal phagocytic cells two days following oral administration. However, no augmentation was observed following intraperitoneal administration.

8. Riker 3-M was effective in augmenting resistance to banzi virus encephalitis when administered orally or intraperitoneally on the day of virus challenge. In contrast, no protection was observed when this drug was administered by gavage on either day one or two post infection.

9. PR 879-317A was effective in enhancing reticuloendothelial system function when given two days prior to assay at a dose of 10 mg/kg. In addition, this drug was still active four days after treatment with a 50 mg/kg dose.

10. PR 879-317A had no effect on macrophage cytotoxicity at any of the dosages or times of administration examined.

11. PR 879-317A was ineffective in augmenting splenic natural killer cell activity when administered at either two or four days prior to assay.

12. PR 879-317A had no effect on phagocytic activity as measured by uptake of fluorescent bacteria.

13. PR 879-317A significantly enhanced resistance to HSV-1 induced pneumonitis when administered intraperitoneally one day after virus challenge. In contrast, the drug administered by the intravenous route was ineffective.

14. PR 879-317A was ineffective in our murine models of hepatitis, influenza and banzi encephalitis.

15. Administration of CGP 31,362 resulted in significant macrophage cytotoxicity.

16. Treatment of mice with CGP 31,362 was effective in enhancing pulmonary immunity by increasing the number of phagocytic cells in the lungs two days after intranasal instillation of drug.

17. When administered by the intranasal route, several days prior to HSV-1 induced pneumonitis, CGP 31,362 therapy resulted in significant protection.

18. CGP 31,362 was not able to enhance resistance to HSV-1 induced hepatitis regardless of the route of administration.

19. Mice receiving exogenous interferon 24 hours prior to assay had augmented reticuloendothelial system function.

20. Peritoneal macrophages obtained one day following intraperitoneal injection of recombinant interferon were cytotoxic.

21. Recombinant interferon did not increase the total number of phagocytic cells but was able to increase the phagocytic activity of peritoneal macrophages obtained one day following intraperitoneal inoculation.

22. When administered intracranially recombinant IFN was able to prolong the mean survival time of banzi virus infected animals but was not able to prevent death.

23. Recombinant interferon given intravenously on the day of banzi virus infection was fully protective.

24. Mice receiving interferon on the day of or one day after infection had markedly reduced virus titers in their brains and spinal cords.

25. CL 246738 was able to protect mice when administered either two, four or six days prior to infection with banzi virus. Half of the banzi virus infected mice were protected when CL 246738 was administered eight days prior to infection. However, no protection was observed when this drug was administered ten days prior to infection.

VI. RECOMMENDATIONS

During this contract period we have examined approximately 25 immunomodulating drugs for their ability to stimulate various components of the specific and nonspecific immune systems, as well as their ability to enhance resistance to influenza, herpes-, arena-, bunya- and flaviviruses. All of these compounds were able to stimulate at least one component of the immune system and some were able to stimulate many. Although there was no correlation between stimulation of a particular component of the immune system and resistance to virus infection, in general, drugs that were most efficacious, appeared to stimulate a broader range of immune functions. The overall results of our experiments are summarized in the following three summary tables (Summary Tables I-III). These tables identify the promising immunomodulators which should be evaluated in non human primate animal models for their immunopotentiating and antiviral capabilities. These drugs include: Poly I:C-LC, (poly riboinosinic:poly ribocytidylic acid complexed with carboxymethyl cellulose and poly-L-lysine); CL 246738, [3,6-bis(2-piperidinoethoxy)acridine trihydrochloride]; Ampligen, (a mismatched analog of poly riboinosinic:poly ribocytydylic acid); the pyrimidinone ABPP, {2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone}; the Riker-3M compound {1-(2-methyl propyl)-1H-imidazo[4,5-c]quinolin 4-amine} and a hybrid recombinant human interferon (rhuIFNα-B/D). Future studies are needed to determine the dosage and scheduling which will be needed to optimize the efficacy of theses drugs in man.

Summary Table I. Enhancement of immune functions.

ENHANCEMENT OF IMMUNE FUNCTIONS									
	CLEARANCE		PHAGOCYTOSIS		NK CYTOTOXICITY		MACROPHAGE	LYMPHOCYTE	RANK
	D-2	D-4	D-2	D-4	D-2	D-4	CYTOTOXICITY	SUBPOPULATIONS	
POLY I:C-LC	+	±	+	+	+	+	+	0	0.8
CL 246738	+	0	+	0	+	+	0	0	0.5
AMPLIGEN	+	0	±	0	+	0	0	0	0.3
PYRIMIDINONE (ABPP)	+	+	±	+	+	0	+	0	0.6
RIKER-3M	+	0	+	ND	+	+	0	0	0.6
RECOMBINANT IFN (rhuIFN α-B/D)	+	0	+	ND	+	ND	+	0	0.7

+ = Significant enhancement of activity.

± = Marginal but consistent enhancement of activity.

0 = Not different from control group.

ND = Not done.

$$\text{Rank} = \frac{\text{Sum of the +'s}}{\text{Total number of assays}}$$

Summary Table II. Enhancement of antiviral functions.

ENHANCEMENT OF ANTIVIRAL ACTIVITIES										
			RESISTANCE							
						HSV-1				
	SERUM INTERFERON	MACROPHAGE EXTRINSIC ANTIVIRAL ACTIVITY	BANZI (DO)	PICHINDE (MULTIPLE)	PUNTA TORO (D-2)	PHEU. (D-2)	HEP. (D-2)	ENCEPH. (D-2)	WLUENZA (D-2)	RANK
POLY I:C-LC	++	++	++	0	++	++	++	0	+	1.4
CL 246738	+	++	+	0	++	++	0	0	0	0.9
AMPLIGEN	+	+	+	0	ND	++	0	ND	0	0.7
PYRIMIDINONE (ABPP)	±	ND	+	ND	ND	0	0	+	0	0.4
RIKER-3M	+	ND	++	ND	ND	0	ND	ND	ND	1.0
RECOMBINANT IFN (rhuIFN α-B/D)	NA	++	++	0	ND	++	0	0	+	0.9

++ = Greater than a two log difference between control and experimental groups for serum interferon or macrophage antiviral activity. Drug affords protection in resistance studies.

+ = Less than two log difference between control and experimental groups for serum interferon or macrophage antiviral activity. Drug increases mean survival time but does not afford protection.

0 = Not different from control group.

ND = Not done

$$\text{Rank} = \frac{\text{Sum of the +'s}}{\text{Total number of assays}}$$

Summary Table III. Overall rank.

OVERALL RANK			
	IMMUNE FUNCTIONS	ANTIVIRAL	OVERALL
POLY I:C-LC	0.8	1.4	2.2
CL 246738	0.5	0.9	1.5
AMPLIGEN	0.3	0.7	1.0
PYRIMIDINONE (ABPP)	0.6	0.4	1.0
RIKER-3M	0.6	1.0	1.6
RECOMBINANT IFN (rhuIFN α-B/D)	0.7	0.9	1.6
TOTAL POSSIBLE	1.0	2.0	3.0

Overall rank is the sum of the ranks from Summary Tables I and II.

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Table 1. Clearance and tissue localization of SRBC following Poly I:C-LC treatment.

TREATMENT		RBC/mg Tissue (x1000)			Phagocytic Index		
		Liver	Spleen	Lung	T/2 (min)	alpha Value	K Value
CONTROL	Mean	78.81	129.47	17.53	3.13	7.40	.1051
	Std. Dev.	11.39	39.73	10.40	.94	1.42	.0351
PolyIC-LC Day -2	Mean	27.99	223.19	74.70	13.40	4.80	.0280
	Std. Dev.	12.48	108.12	33.50	6.44	1.01	.0150
	P-Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PolyIC-LC Day -4	Mean	64.06	140.61	46.81	3.73	6.70	.0936
	Std. Dev.	23.53	52.02	23.38	1.85	1.64	.0319
	P-Value	<0.01	N.S.	<0.001	N.S.	N.S.	N.S.
PolyIC-LC Day -7	Mean	87.57	62.66	15.07	2.76	6.19	.1216
	Std. Dev.	14.11	15.66	11.44	.98	.85	.0389
	P-Value	<0.05	<0.001	<0.005	N.S.	<0.01	N.S.
PolyIC-LC Day -14	Mean	74.61	119.29	31.31	3.44	7.26	.1005
	Std. Dev.	12.82	39.55	13.17	1.37	.70	.0380
	P-Value	N.S.	N.S.	<0.001	N.S.	N.S.	N.S.

Poly I:C-LC (5 mg/kg) was injected iv. on day -2, -4, -7 or -14 and tested for clearance on day 0.

N.S. = Not significant

Table 2. Clearance and tissue localization of SRBC following oral CL 246738 treatment.

TREATMENT		RBC/mg Tissue (x1000)			Phagocytic Index		
		Liver	Spleen	Lung	T/2 (min)	alpha Value	K Value
CONTROL	Mean	73.08	187.46	34.83	4.83	6.54	.0706
	Std. Dev.	10.81	60.45	19.56	1.64	.71	.0286
CL 246738 Day -2	Mean	95.08	121.11	10.60	2.43	7.45	.1283
	Std. Dev.	15.16	36.00	6.58	.47	.91	.0256
	p-Value	<0.001	<0.001	<0.001	<0.001	<0.005	<0.001
CL 246738 Day -4	Mean	88.23	103.35	15.64	3.05	7.14	.1152
	Std. Dev.	19.48	37.54	11.94	1.12	.77	.0462
	P-Value	<0.02	<0.001	<0.005	<0.005	<0.05	<0.005
CL 246738 Day -7	Mean	96.39	200.88	46.75	5.04	7.08	.0642
	Std.Dev.	18.66	62.69	12.92	1.24	.7098	.0226
	p-Value	<0.005	N.S.	N.S.	N.S.	N.S.	N.S.
CL 246738 Day -14	Mean	90.85	201.51	37.30	4.59	7.21	.0672
	Std.Dev.	12.08	46.86	7.65	.75	.2760	.0124
	p-Value	<0.005	N.S.	N.S.	N.S.	N.S.	N.S.

CL 246738 (200 mg/kg) was administered orally on day -2, -4 , -7 or -14 and tested for clearance on day 0.

N.S. = Not significant

Table 3. Clearance and tissue localization of SRBC following Ampligen treatment.

TREATMENT		RBC/mg Tissue (x1000)			Phagocytic Index		
		Liver	Spleen	Lung	T/2 (min)	alpha Value	K Value
CONTROL	Mean	72.38	172.22	25.02	3.82	6.60	.0842
	Std. Dev.	12.50	41.66	11.35	1.10	.70	.0228
Ampligen Day -2	Mean	95.03	115.00	15.08	2.25	7.38	.1370
	Std. Dev.	11.15	50.67	10.47	.46	.48	.0321
	p-Value	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001
CONTROL	Mean	70.94	157.07	28.53	3.88	6.69	.0847
	Std. Dev.	13.31	44.00	19.39	1.24	.89	.0266
Ampligen Day -4	Mean	84.17	130.14	21.50	3.31	6.52	.1443
	Std. Dev.	15.54	52.13	13.33	1.19	.74	.1658
	P-Value	<0.02	N.S.	N.S.	N.S.	N.S.	N.S.
CONTROL	Mean	75.65	157.81	21.86	3.46	6.69	.0893
	Std. Dev.	12.86	41.50	10.30	.75	.85	.0193
Ampligen Day -7	Mean	84.59	151.59	26.47	3.68	6.95	.0841
	Std. Dev.	7.53	53.83	8.55	.68	.80	.0149
	P-Value	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Ampligen (5 mg/kg) was injected iv. on day -2, -4 or -7 and tested for clearance on day 0.

N.S. = Not significant

Table 4. Clearance and tissue localization of SRBC following iv. CL 246738 treatment.

TREATMENT		RBC/mg Tissue (x1000)			Phagocytic Index		
		Liver	Spleen	Lung	T/2 (min)	alpha Value	K Value
CONTROL	Mean	74.82	131.77	23.68	3.90	6.52	.0910
	Std. Dev.	12.12	65.71	17.67	1.65	.84	.0378
CL 246738 Day -2	Mean	84.19	129.87	14.55	3.16	6.91	.1032
	Std. Dev.	10.23	56.17	16.19	1.00	.59	.0286
	P-Value	<0.05	N.S.	N.S.	N.S.	N.S.	N.S.
CONTROL	Mean	71.22	126.01	25.58	4.02	6.39	.0882
	Std. Dev.	11.25	62.73	19.44	1.73	.89	.0369
CL 246738 Day -4	Mean	70.86	157.14	26.74	3.79	6.64	.0755
	Std. Dev.	7.56	27.04	17.69	.81	.85	.0152
	P-Value	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

CL 246738 (25 mg/kg) was injected iv. on day -2 or -4 and tested for clearance on day 0.

N.S. = Not significant

Table 5. Effect of Poly I:C-LC on antibody plaque forming cell (PFC) response.

Treatment		Cells/ Spleen x1000	IgM Plaques		IgG Plaques	
			PFC/10^6	PFC/Spl	PFC/10^6	PFC/Spl
Control	Geo. Mean	79	656	47,835	861	62,732
	Log Mean	1.86	2.82	4.68	2.94	4.80
	Std. Dev.	0.18	0.24	0.30	0.43	0.42
	Geo. Mean	131	412	42,493	141	19,184
PolyI:C-LC	Log Mean	2.12	2.62	4.63	2.15	4.28
Day -1	Std. Dev.	0.26	0.35	0.43	0.37	0.46
	p-Value	<0.001	<0.01	N.S.	<0.001	<0.001
	Geometric	102	744	75,660	954	97,237
PolyI:C-LC	Log Mean	2.01	2.87	4.88	2.98	4.99
Day +1	Std. Dev.	0.16	0.25	0.29	0.40	0.45
	p-Value	<0.01	N.S.	<0.05	N.S.	N.S.

Mice were injected iv. with Poly I:C-LC (5 mg/kg) either 1 day before or 1 day after i.p. immunization with $1x10^8$ SRBC and tested 5 days later. Results from 4 experiments were pooled for analysis.

N.S. = Not significant

Table 6. Effect of CL 246738 on antibody plaque forming cell (PFC) response.

Treatment		Cells/ Spleen x1000	IgM Plaques		IgG Plaques	
			PFC/10^6	PFC/Spl	PFC/10^6	PFC/Spl
Control	Geo. Mean	86	894	76,779	1,183	101,625
	Log Mean	1.93	2.95	4.89	3.07	5.01
	Std. Dev.	0.17	0.11	0.20	0.25	0.37
	Geo. Mean	88	975	85,566	592	51,971
CL 246738	Log Mean	1.94	2.99	4.93	2.77	4.72
Day -1	Std. Dev.	0.12	0.18	0.17	0.28	0.32
	p-Value	N.S.	N.S.	N.S.	<0.05	N.S.
	Geometric	95	1,027	97,513	1,101	104,506
CL 246738	Log Mean	1.98	3.01	4.99	3.04	5.02
Day +1	Std. Dev.	0.09	0.11	0.17	0.37	0.41
	p-Value	<0.001	N.S.	N.S.	N.S.	N.S.

Mice were given CL 246738 (200 mg/kg) either 1 day before or 1 day after i.p.immunization with $1x10^8$ SRBC tested 5 days later. Results from 2 experiments were pooled for analysis.

N.S. = Not significant

Table 7. Effect of Ampligen on antibody plaque forming cell (PFC) response.

Treatment		Cells/ Spleen x1000	IgM Plaques		IgG Plaques	
			PFC/10^6	PFC/Spl	PFC/10^6	PFC/Spl
Control	Geo. Mean	84	664	56,062	1,973	166,484
	Log Mean	1.93	2.82	4.75	3.30	5.22
	Std. Dev.	0.12	0.17	0.16	0.15	0.12
Ampligen Day -1	Geo. Mean	164	616	100,935	1,216	157,984
	Log Mean	2.14	2.79	5.00	3.09	5.20
	Std. Dev.	0.06	0.24	0.25	0.18	0.38
	p-Value	<0.001	N.S.	<0.05	<0.02	N.S.
Ampligen Day +1	Geometric	212	566	120,352	2,575	547,473
	Log Mean	2.33	2.75	5.08	3.41	5.74
	Std. Dev.	0.10	0.16	0.14	0.17	0.09
	p-Value	<0.001	N.S.	<0.001	N.S.	<0.001

Mice were injected iv. with Ampligen (5 mg/kg) either 1 day before or 1 day after i.p. immunization with $1x10^8$ SRBC tested 5 days later. Results from 2 experiments were pooled for analysis.

N.S. = Not significant

Table 8. Effect of Poly I:C-LC on spleen and liver NK cytotoxicity.

	PERCENT CYTOTOXICITY					
	Spleen at E:T Ratios			Liver at E:T Ratios		
Treatment	100:1	50:1	25:1	100:1	50:1	25:1
Control	25	17	10	Not Tested		
Poly I:C-LC Day -2*	57	38	25			
Poly I:C-LC Day -4*	40	29	19			
Control	18	10	5	12	10	9
Poly I:C-LC Day -7$	25	13	3	50	54	50
Poly I:C-LC Day -14$	18	12	5	40	31	27

Poly I:C-LC (5 mg/kg) was injected iv. on day -2, -4, -7 or -14 and tested for NK cytotoxicity on day 0.

*p-values <0.02 relative to control.

$p- values <0.02 relative to control for liver cells only. Spleen cels from drug treated animals were not significantly different from controls.

Table 9. Effect of CL 246738 on spleen and liver NK cytotoxicity.

Treatment		PERCENT CYTOTOXICITY					
		Spleen at E:T Ratios			Liver at E:T Ratios		
		100:1	50:1	25:1	100:1	50:1	25:1
Day -2	Control	7	5	5	8	7	5
	CL-246738*	57	41	27	12	11	8
Day -4	Control	20	14	9	2	2	1
	CL-246738*	65	60	49	15	12	9

CL 246738 (200 mg/kg) was given orally on day -2 or -4 and tested for NK cytotoxicity on day 0.

*p-values <0.02 relative to control.

Table 10. Effect of Ampligen on spleen and liver NK cytotoxicity.

Treatment	PERCENT CYTOTOXICITY					
	Spleen at E:T Ratios			Liver at E:T Ratios		
	100:1	50:1	25:1	100:1	50:1	25:1
Control	19	14	12	9	8	7
Ampligen Day -2*	43	30	18	15	15	11
Ampligen Day -4	33	22	13	13	16	13

Ampligen (5 mg/kg) was injected iv. on day -2 or -4 and tested for NK cytotoxicity on day 0.

*p-values <0.02 relative to control for spleen. Liver cells from drug treated animals were not significantly different from controls.

Table 11. Effect of Poly I:C-LC and CL 246738 on the induction of specific T cell cytotoxicity in splenocytes.

Treatment	% Cytotoxicity at E:T Ratios			
	100:1	50:1	25:1	12.5:1
Control	63	48	30	17
Poly I:C-LC Day -2*	24	9	6	3
Control	35	24	16	9
CL 246738 Day -2	27	18	9	4

Poly I:C-LC (5 mg/kg) was injected iv. or CL 246738 (200 mg/kg) was administered orally on day -2 or -4 and tested on day 0 for *in vitro* induction of T cell cytotoxicity against allogeneic target.

*p-values <0.01 relative to control.

Table 12. Effects of Poly I:C-LC, CL 246738 and Ampligen on splenic lymphocyte subpopulations.

Treatment	% of Population				
	B Cells	T Cells	Ly 1+2+ Cells	Ly 1+2- Cells	Ly 1-2+ Cells
Control	51 ± 12	31 ± 10	7 ± 5	16 ± 9	4 ± 2
Poly I:C-LC Day -2 (#)	62 ± 2	33 ± 9	7 ± 3	15 ± 7	0 ± 1
Poly I:C-LC Day -4 (#)	42 ± 13	36 ± 11	6 ± 1	18 ± 10	4 ± 4
Poly I:C-LC Day -7 (*)	31	17	2	6	4
Poly I:C-LC Day -14 (#)	55 ± 10	32 ± 1	6 ± 6	16 ± 2	3 ± 4
CL 246738 Day -2 (#)	57 ± 13	27 ± 7	6 ± 8	13 ± 3	9 ± 4
CL 246738 Day -4 (*)	62	44	15	11	0
CL 246738 Day -7 (*)	40	17	0	11	6
Ampligen Day -2 (*)	35	18	3	7	1
Ampligen Day -4 (*)	36	20	2	8	3

Poly I:C-LC or Ampligen (5 mg/kg) was injected iv. or CL 246738 (200 mg/kg) was administered orally on day -2, -4 or -7 and tested on day 0.

(#) Differences are not significant
(*) Single experiment.

Table 13. Effects of CL 246738 and Ampligen on macrophage antiviral activity.

Treatment	Virus Titer/ml	Log Reduction
None	1.9×10^7	-
Control	3.3×10^5	1.76
CL 246738	4.7×10^3	3.61
Ampligen	2.5×10^4	2.88
Poly I:C-LC	Not Tested	-

CL 246738 (200 mg/kg) was administered orally or Ampligen (5 mg/kg) was injected iv. on day -2 days and tested on day 0.

Table 14. Clearance and tissue localization of SRBC following treatment with various pyrimidinones on day -2.

Treatment		RBC/mg Tissue (x1000)			Phagocytic Index		
		Spleen	Liver	Lung	T/2 (min)	alpha Value	K Value
CMC Control	Mean	148	81	20	3.78	7.32	.0913
	Std. Dev.	24	15	12	1.31	1.28	.0294
ABPP	Mean	103	90	7	1.88	7.86	.1635
	Std. Dev.	37	16	4	.30	.73	.0242
	P-Value	<0.001	NS	<0.001	<0.001	NS	<0.001
ACPP	Mean	100	93	8	3.14	7.33	.1153
	Std. Dev.	30	26	7	1.78	1.46	.0433
	P-Value	<0.001	NS	<0.02	NS	NS	NS
AIPP	Mean	105	104	15	2.28	8.14	.1399
	Std. Dev.	40	15	8	.60	.93	.0325
	P-Value	<0.001	<0.001	NS	<0.005	NS	<0.001
ABMFPP	Mean	63	105	5	2.13	7.51	.1542
	Std. Dev.	23	22	3	.71	.70	.0435
	P-Value	<0.001	<0.005	<0.005	<0.005	NS	<0.001
ACDFPP	Mean	98	87	16	2.66	8.28	.1235
	Std. Dev.	33	25	12	.94	.66	.0338
	P-Value	<0.001	NS	NS	<0.05	<0.05	<0.01
Saline	Mean	149	74	24	4.82	6.47	.0707
	Std. Dev.	43	14	13	1.81	.81	.0257
	P-Value	NS	NS	NS	<0.01	<0.005	<0.01

Pyrimidinones (250 mg/kg) were given intraperitoneally in 1% carboxymethyl- cellulose (CMC) two days before assay. All results are compared with those obtained with the CMC control. A saline control group was also included.

ns = Not significant

Table 15. Clearance and tissue localization of SRBC following treatment with various pyrimidinones on day -4.

Treatment		RBC/mg Tissue (x1000)			Phagocytic Index		
		Spleen	Liver	Lung	T/2 (min)	alpha Value	K Value
CMC Control	Mean	124	97	20	2.96	7.51	.1129
	Std. Dev.	45	31	28	1.15	.96	.0336
ABPP	Mean	116	85	10	2.00	7.86	.1528
	Std. Dev.	40	11	6	.31	.74	.0193
	P-Value	NS	NS	NS	<0.02	NS	<0.005
ACPP	Mean	114	99	8	2.45	7.57	.1300
	Std. Dev.	47	16	5	.63	.90	.0314
	P-Value	NS	NS	NS	NS	NS	NS
AIPP	Mean	75	109	8	2.25	7.91	.1411
	Std. Dev.	31	23	4	.60	.68	.0328
	P-Value	<0.01	NS	NS	NS	NS	<0.05
ABMFPP	Mean	95	103	6	2.28	7.25	.1079
	Std. Dev.	55	20	5	.85	.59	.0458
	P-Value	<0.05	NS	<0.05	<0.05	NS	<0.005
ACDFPP	Mean	89	102	19	2.48	7.67	.1286
	Std. Dev.	43	14	15	.62	.94	.0328
	P-Value	<0.05	NS	NS	NS	NS	NS
Saline	Mean	148	83	20	3.94	6.82	.0841
	Std. Dev.	60	21	11	1.35	.72	.0276
	P-Value	NS	<0.05	NS	<0.005	<0.005	<0.001

Pyrimidinones (250 mg/kg) were given intraperitoneally in 1% carboxymethyl- cellulose (CMC) 4 days before assay. All results are compared with those obtained with the CMC control. A saline control group was also included.

NS = Not significant

Table 16. Activation of cytotoxic macrophages by pyrimidinones.

Treatment	PERCENT CYTOTOXICITY					
	Experiment No. 1			Experiment No. 2		
	40:1	20:1	10:1	40:1	20:1	10:1
ABPP	88#	65*	38	99@	96@	51*
ACPP	97#	53	-28	65#	55@	58*
AIPP	21	31	31	21	34	31
ABMFPP	74#	59@	60*	86	31	-42
ACDFPP	19	5	8	17	31	20

Pyrimidinones (250 mg/kg) were given intraperitoneally in 1% carboxymethyl- cellulose (CMC) 4 days before assay. Control mice were give CMC alone. Percent cytotoxicity was calculated as follows:

$$\frac{C-T}{T} \times 100$$

Where, C= Counts per Minute (CPM) in cultures with macrophages from CMC treated mice and T= CPM in cultures from pyrimidinone treated mice.

* $p < 0.05$

# $p < 0.01$

@ $p < 0.001$

Table 17. PGE_2 secretion by peritoneal macrophages from ABPP treated mice.

Treatment	pg Prostaglandin E_2 per mg Protein			
	Experiment No.1		Experiment No.2	
	Mean (S.D.)	p	Mean (S.D.)	p
CMC Control	369.2 (12.2)	-	214.5 (49.3)	-
ABPP Day -2	45.3 (2.3)	<0.001	30.2 (11.7)	<0.005
CMC Control	109.0 (27.7)	-	126.6 (20.8)	-
ABPP Day -4	24.1 (2.6)	<0.01	37.1 (11.9)	<0.005
CMC Control	76.9 (3.3)	-	73.4 (4.0)	-
ABPP Day -7	8.3 (1.9)	<0.001	12.2 (2.5)	<0.001
CMC Control	51.1 (14.2)	-	221.7 (73.2)	-
ABPP Day -14	62.1 (46.2)	NS	123.7 (78.1)	NS

Mice were treated intraperitoneally with ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), on various days before assaying for the secretion of PGE-2 by adherent peritoneal exudate cells over a 20 hour time period. PGE-2 levels were determined by radioimmunoassay. Protein content of the adherent cells was determined after lysing a duplicate sample of adherent cells by freezing and thawing three times.

NS = Not significant

Table 18. PGE_2 secretion by peritoneal macrophages from ACPP treated mice.

Treatment	pg Prostaglandin E_2 per mg Protein			
	Experiment No.1		Experiment No.2	
	Mean (S.D.)	p	Mean (S.D.)	p
CMC Control	215.0 (55.1)	-	415.1 (104.2)	NS
ACPP Day -2	26.9 (18.8)	<0.02	25.9 (8.9)	<0.005
CMC Control	50.9 (7.0)	-	23.4 (3.3)	-
ACPP Day -4	8.9 (4.2)	<0.001	13.3 (2.1)	<0.02
CMC Control	81.9 (43.0)	-	40.2 (15.7)	-
ACPP Day -7	16.9 (4.7)	<0.05	7.8 (2.0)	<0.025
CMC Control	43.9 (6.7)	-	51.1 (14.2)	-
ACPP Day -14	8.5 (1.3)	<0.001	51.3 (45.4)	NS

Mice were treated intraperitoneally with ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), on various days before assaying for the secretion of PGE-2 by adherent peritoneal exudate cells over a 20 hour time period. PGE-2 levels were determined by radioimmunoassay. Protein content of the adherent cells was determined after lysing a duplicate sample of adherent cells by freezing and thawing three times.

NS = Not significant

Table 19. PGE_2 secretion by peritoneal macrophages from AIPP treated mice.

Treatment	pg Prostaglandin E_2 per mg Protein			
	Experiment No.1		Experiment No.2	
	Mean (S.D.)	p	Mean (S.D.)	p
CMC Control	283.2 (46.6)	-	215.0 (55.1)	-
AIPP Day -2	27.2 (11.1)	<0.001	39.0 (16.1)	<0.02
CMC Control	50.9 (7.0)	-	23.4 (3.3)	-
AIPP Day -4	8.2 (2.1)	<0.001	9.0 (2.9)	<0.005
CMC Control	81.9 (43.0)	-	73.4 (4.0)	-
AIPP Day -7	11.7 (2.1)	<0.05	11.7 (1.4)	<0.001
CMC Control	43.9 (6.7)	-	51.1 (14.2)	-
AIPP Day -14	10.1 (1.9)	<0.005	8.0 (3.1)	<0.05

Mice were treated intraperitoneally with AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), on various days before assaying for the secretion of PGE-2 by adherent peritoneal exudate cells over a 20 hour time period. PGE-2 levels were determined by radioimmunoassay. Protein content of the adherent cells was determined after lysing a duplicate sample of adherent cells by freezing and thawing three times.

NS = Not significant

Table 20. PGE_2 secretion by peritoneal macrophages from ABMFPP treated mice.

Treatment	pg Prostaglandin E_2 per mg Protein			
	Experiment No.1		Experiment No.2	
	Mean (S.D.)	p	Mean (S.D.)	p
CMC Control	81.7 (31.1)	-	106.4 (27.0)	-
ABMFPP Day -2	18.2 (17.3)	<0.05	27.2 (2.7)	<0.01
CMC Control	50.9 (7.0)	-	23.4 (3.3)	-
ABMFPP Day -4	9.5 (3.4)	<0.001	7.6 (0.4)	<0.005
CMC Control	81.9 (43.0)	-	40.2 (15.7)	-
ABMFPP Day -7	8.5 (0.8)	<0.05	4.1 (0.5)	<0.02
CMC Control	43.9 (6.7)	-	51.1 (14.2)	-
ABMFPP Day -14	90.0 (45.0)	<0.02	8.1 (2.4)	<0.01

Mice were treated intraperitoneally with ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), on various days before assaying for the secretion of PGE-2 by adherent peritoneal exudate cells over a 20 hour time period. PGE-2 levels were determined by radioimmunoassay. Protein content of the adherent cells was determined after lysing a duplicate sample of adherent cells by freezing and thawing three times.

Table 21. PGE_2 secretion by peritoneal macrophages from ACDFPP treated mice.

Treatment	pg Prostaglandin E_2 per mg Protein			
	Experiment No.1		Experiment No.2	
	Mean (S.D.)	p	Mean (S.D.)	p
CMC Control	81.7 (31.1)	-	106.4 (27.0)	-
ACDFPP Day -2	9.5 (2.4)	<0.02	31.6 (3.5)	<0.01
CMC Control	50.9 (7.0)	-	23.4 (3.3)	-
ACDFPP Day -4	6.1 (1.9)	<0.001	10.7 (2.1)	<0.005
CMC Control	81.9 (43.0)	-	73.4 (4.0)	-
ACDFPP Day -7	12.0 (4.4)	<0.05	5.8 (2.1)	<0.001
CMC Control	43.9 (6.7)	-	51.1 (14.2)	-
ACDFPP Day -14	76.7 (20.5)	NS	4.4 (1.5)	<0.005

Mice were treated intraperitoneally with ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), on various days before assaying for the secretion of PGE-2 by adherent peritoneal exudate cells over a 20 hour time period. PGE-2 levels were determined by radioimmunoassay. Protein content of the adherent cells was determined after lysing a duplicate sample of adherent cells by freezing and thawing three times.

NS = Not significant

Table 22. Clearance and tissue localization of SRBC following treatment with AM-3.

Treatment		Phagocytic Index			RBC/mg Tissue (x1000)		
		K Value	alpha Value	T/2 (min)	Spleen	Liver	Lung
Control	Mean	0.0824	6.38	3.82	132	80	20
	Std. Dev.	0.0179	0.82	0.91	36	11	10
AM-3 Day -2	Mean	0.1369	7.30	2.26	101	106	5
	Std. Dev.	0.0225	0.55	0.39	32	18	3
	P-Value	<0.001	<0.01	<0.001	NS	<0.005	<0.001
AM-3 Day -4	Mean	0.0854	5.94	3.65	132	86	18
	Std. Dev.	0.0188	0.57	0.68	33	14	6
	P-Value	NS	NS	NS	NS	NS	NS

Mice were injected with AM-3 (400 mg/kg) intraperitoneally, two or four days before testing for clearance of sheep erythrocytes (SRBC) from circulation and their localization in the various organs. Control mice received the same volume of pyrogen-free saline. The K value represents the rate of clearance and $t_{1/2}$ represents the time to clear half of the material from the circulation. Both values were obtained by plotting the concentration of injected material remaining in circulation at 2, 4, 6, and 10 minutes after injection.

NS = Not significant

Table 23. Clearance and tissue localization of SRBC following treatment with Riker 3M.

TREATMENT		Phagocytic Index			RBC/mg Tissue (x1000)		
		K Value	alpha Value	T/2 (min)	Spleen	Liver	Lung
Control Lactic Acid	Mean	0.1343	7.50	2.31	114	89	7
	Std. Dev.	0.0297	0.65	0.49	23	6	3
Riker 3M Day -2	Mean	0.1840	7.98	1.64	96	101	3
	Std. Dev.	0.0113	0.27	0.10	9	8	1
	P-Value	<0.01	NS	<0.02	NS	<0.05	<0.05
Riker 3M Day -4	Mean	0.1079	6.35	2.93	143	82	19
	Std. Dev.	0.0295	0.78	0.91	40	8	14
	P-Value	NS	NS	NS	NS	NS	NS

Mice were injected with Riker 3M (10 mg/kg) in 1% lactic acid, intraperitoneally, two or four days before testing for clearance of sheep erythrocytes (SRBC) from circulation and their localization in th various organs. Control mice received the same volume of 1% lactic acid. The K value represents the rate of clearance and $t_{1/2}$ represents the time to clear half of the material from the circulation. Both values were obtained by plotting the concentration of injected material remaining in circulation at 2, 4, 6, and 10 minutes after injection.

NS = Not significant

Table 24. Clearance and tissue localization of SRBC following intravenous treatment with PR 879-317A (10 mg/kg).

Treatment		RBC/mg Tissue (x1000)			Phagocytic Index		
		Spleen	Liver	Lung	T/2 (min)	alpha Value	K Value
Control	Mean	77.62	55.49	37.13	6.21	4.84	.0523
	Std. Dev.	65.57	13.27	22.22	1.72	.61	.0161
PR 879-317A Day -2	Mean	67.59	72.59	26.25	3.97	5.46	.0775
	Std. Dev.	65.03	5.30	14.63	.56	.56	.0139
	P-Value	NS	<0.005	NS	<0.005	<0.05	<0.005
Control	Mean	130.75	46.81	53.31	6.91	5.22	.0448
	Std. Dev.	49.74	9.88	19.21	1.32	.59	.0079
PR 879-317A Day -4	Mean	192.58	68.73	53.91	5.99	5.70	.0513
	Std. Dev.	44.42	8.00	16.88	.91	.31	.0088
	P-Value	NS	<0.01	NS	NS	NS	NS

Mice were given PR 879-317A (10 mg/kg) intravenously, 2 or 4 days before testing for clearance of chromium labelled sheep erythrocytes from circulation and their localization in spleen, liver and lung.

NS = Not significant

Table 25. Clearance and tissue localization of SRBC following intraperitoneal treatment with PR 879-317A (50 mg/kg).

		RBC/mg Tissue (x1000)			Phagocytic Index		
		Spleen	Liver	Lung	T/2 (min)	alpha Value	K Value
Control	Mean	139.18	52.94	27.10	5.37	6.26	.0564
	Std. Dev.	70.60	22.41	20.74	.42	.64	.0043
PR 879-317A Day -2	Mean	107.20	93.25	14.64	2.94	8.94	.1051
	Std. Dev.	42.18	16.15	2.34	.52	.55	.0172
	P-Value	NS	<0.02	NS	<0.001	<0.001	<0.001
PR 879-317A Day -4	Mean	167.92	91.91	34.87	3.74	7.76	.0812
	Std. Dev.	71.14	13.44	15.57	.38	.35	.0090
	P-Value	NS	<0.02	NS	<0.001	<0.005	<0.005

Mice were given PR 879-317A (50 mg/kg) intraperitoneally, 2 or 4 days before testing for clearance of chromium labelled sheep erythrocytes from circulation and their localization in spleen, liver and lung.

NS = Not significant

Table 26. Clearance and tissue localization of SRBC following intravenous treatment with isoprinosine.

TREATMENT		RBC/mg Tissue (x1000)			Phagocytic Index		
		Spleen	Liver	Lung	T/2 (min)	alpha Value	K Value
Control	Mean	126.18	76.77	20.63	3.57	7.18	.0892
	Std. Dev.	27.98	12.45	8.86	.94	1.23	.0214
Isoprinosine Day- 2	Mean	128.21	84.22	19.28	3.58	6.53	.0890
	Std. Dev.	47.55	13.09	10.14	.89	.80	.0227
	P-Value	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Isoprinosine Day- 4	Mean	131.29	94.59	18.51	3.64	7.12	.0905
	Std. Dev.	27.91	22.86	8.36	1.06	.90	.0312
	P-Value	N.S.	<0.05	N.S.	N.S.	N.S.	N.S.
Isoprinosine Day- 7	Mean	119.37	102.49	17.70	3.26	7.45	.0978
	Std. Dev.	32.21	14.15	5.93	.96	.69	.0221
	P-Value	N.S.	<0.001	N.S.	N.S.	N.S.	N.S.

Mice were given Isoprinosine (50 mg/kg) intravenously, 2, 4 or 7 days before testing for clearance of chromium labelled sheep erythrocytes from circulation and their localization in spleen, liver and lung.

p-Values were obtained by comparison with saline control.

N.S. = Not significant

Table 27. Clearance and tissue localization of SRBC following treatment with Poly I:C-LC or Interferon (rhuIFNα-B/D).

Treatment		Phagocytic Index			RBC/mg Tissue (x1000)		
		K Value	alpha Value	T/2 (min)	Spleen	Liver	Lung
Control	Mean	0.0829	6.41	3.75	172	89	71
	Std. Dev.	0.0140	0.42	0.79	37	14	61
PolyIC-LC i.v.	Mean	0.1267	7.29	2.40	129	108	47
	Std. Dev.	0.0124	0.56	0.23	20	14	26
	P-Value	<0.001	<0.001	<0.001	<0.005	<0.01	NS
IFN i.v.	Mean	0.1339	6.96	2.27	124	112	38
	Std. Dev.	0.0156	0.42	0.23	21	12	29
	P-Value	<0.001	<0.01	<0.001	<0.005	<0.005	NS
IFN i.p.	Mean	0.1446	7.08	2.11	105	105	23
	Std. Dev.	0.0214	0.41	0.31	20	14	19
	P-Value	<0.001	<0.005	<0.001	<0.005	<0.025	NS

Mice were injected with Poly I:C-LC (0.5 mg.kg), intraperitoneally (i.p) or with $1x10^6$ international units, intravenously (i.v.) in 0.2 ml pyrogen-free saline 2 days before test for clearance of sheep erythrocytes (SRBC) from circulation and its localization in the various organs. Control mice received the same volume of pyrogen-free saline. K value represents the rate of clearance and $t_{1/2}$ represents the time to clear half the injected material from circulation. Both values were obtained by plotting the concentration of injected material remaining in circulation at 2, 4, 6, and 10 minutes after injection.

Table 28. Activation of cytotoxic macrophages by Riker-3M compound S26308 (R-837).

Treatment		Oral treatment			I.P. treatment		
		Effector : target ratio			Effector : target ratio		
		40:1	20:1	10:1	40:1	20:1	10:1
Control	CPM	52417	116232	156680	70590	156553	158854
Riker-3M Day -2	CPM	51809	162820	160087	48331	153099	157185
	C.I.	1	-40	-1	31	2	1
	P-value	NS	NS	NS	NS	NS	NS
Riker-3M Day -4	CPM	84775	152734	161177	26197	125760	152562
	C.I.	-61	1	-1	62	20	4
	P-value	NS	NS	NS	<0.025	NS	NS

Target cells alone: 149125 CPM

Mice were given drug (10 mg/kg) in 0.2 ml 1% lactic acid orally or intraperitoneally (i.p.) 2 days before harvesting peritoneal cells. Control mice received the same volume of 1% lactic acid in pyrogen free saline. Peritoneal cells were adhered to microtiter plates, nonadherent cells washed away and $4x10^3$ P388 leukemia cells added to the plate. Cytotoxicity was assayed 48 hours later by pulsing the cells with ^{3}H-thymidine for 16 hours. In this assay effector cells alone do not incorporate any thymidine. Cytotoxicity index (CI) was calculated by applying the formula:

$$100 \times \frac{\text{CPM in control cultures} - \text{CPM in treated cultures}}{\text{CPM in control cultures}}$$

This assay measures both cytolytic and cytostatic effector functions.

NS = Not significantly different from control group by Student's t-test.

Table 29. Lack of activation of cytotoxic macrophages by PR 879-317A.

Treatment	Statistics	Effector to target ratio		
		40:1	20:1	10:1
Control	CPM	843447	1041327	1045222
PR 879-317A	CPM	1039740	972224	921284
	C.I.	-23	7	12
	P-value	N.S.	N.S.	N.S.
Target cells alone:		5078273 CPM		

Mice were injected ip with PR 879-317A (50 mg/kg) in 0.2 ml pyrogen-free saline 2 days or 4 days before test. Control mice received the same volume of pyrogen-free saline. Peritoneal cells were adhered to microtiter plates, nonadherent cells washed away and $4x10^3$ P388 leukemia cells added to the plate. Cytotoxicity was assayed 48 hours later by pulsing the cells with ^{3}H-thymidine for 16 hours. In this assay effector cells alone do not incorporate any thymidine. Cytotoxicity index (CI) was calculated by applying the formula:

$$100 \times \frac{\text{CPM in control cultures - CPM in treated cultures}}{\text{CPM in control cultures}}$$

This assay measures both cytolytic and cytostatic effector functions.

N.S. = Not significant

Table 30. Activation of cytotoxic macrophages by CGP 31,362.

Treatment	Statistics	Effector to target ratio		
		40:1	20:1	10:1
Control	CPM	154468	160068	165274
CGP 31,362	CPM	53456	129200	158063
	C.I.	65	19	4
	P-value	<0.05	<0.025	N.S.
Target cells alone:		151413 CPM		

Mice were injected ip. with CGP 31,362 (0.1 mg/kg) in 0.2 ml pyrogen-free saline 2 days or 4 days before test. Control mice received the same volume of pyrogen-free saline. Peritoneal cells were adhered to microtiter plates, nonadherent cells washed away and $4x10^3$ P388 leukemia cells added to the plate. Cytotoxicity was assayed 48 hours later by pulsing the cells with ^{3}H-thymidine for 16 hours. In this assay effector cells alone do not incorporate any thymidine. Cytotoxicity index (CI) was calculated by applying the formula:

$$100 \times \frac{\text{CPM in control cultures - CPM in treated cultures}}{\text{CPM in control cultures}}$$

This assay measures both cytolytic and cytostatic effector functions.

N.S. = Not significant

Table 31. Activation of cytotoxic macrophages by Poly I:C-LC and rhuIFNα-B/D

Treatment	Statistics	Effector to target ratio 40:1	20:1	10:1
Control	CPM	154468	160068	165274
Poly I:C-LC	CPM	53456	146863	143325
	C.I.	64	8	13
	P-value		N.S.	N.S.
Interferon	CPM	1288	130436	158063
	C.I.	99	8	13
	P-value		N.S.	N.S.
Target cells alone:		151413 CPM		

Mice were injected ip with Poly I:C-LC (0.5 mg/kg) or $1x10^6$ international units in 0.2 ml pyrogen-free saline 2 days before harvesting peritoneal cells. Control mice received the same volume of pyrogen-free saline. Peritoneal cells were adhered to microtiter plates, nonadherent cells washed away and $4x10^3$ P388 leukemia cells added to the plate. Cytotoxicity was assayed 48 hours later by pulsing the cells with ^{3}H-thymidine for 16 hours. In this assay effector cells alone do not incorporate any thymidine. Cytotoxicity index (CI) was calculated by applying the formula:

$$100 \times \frac{\text{CPM in control cultures} - \text{CPM in treated cultures}}{\text{CPM in control cultures}}$$

This assay measures both cytolytic and cytostatic effector functions.

N.S. = Not significant

Table 32. Splenic NK cytotoxicity following oral or intraperitoneal administration of 10 mg/kg of Riker 3M.

Treatment	% Cytotoxicity		
	Effector to target ratio		
	100:1	50:1	25:1
Saline control	9	6	4
1% lactic acid (oral)	10	6	5
Riker 3M (oral)	29*	19*	12*
1% lactic acid i.p.	13	9	7
Riker 3M i.p.	25*	14*	9*

Mice received either oral or intraperitoneal doses of Riker 3M two days prior to removal of spleens and analysis of NK cytotoxicity. YAC cells were used as targets and 4 mice per treatment group were analyzed. The assay is based on the release of chromium from labeled cells. Percent toxicity was calculated using the formula:

$$100 \times \frac{\text{cpm release in test} - \text{spontaneous release}}{\text{maximum cpm release} - \text{spontaneous release}}$$

* $p<0.05$

Table 33. Effect of PR 879-317A on splenic natural killer cells

Treatment	% Cytotoxicity Effector to target ratio		
	100:1	50:1	25:1
Control	15	16	12
PR 879-317A Day -2	14	15	13
PR 879-317A Day -4	12	14	12
Control	31	13	9
Isoprinosine Day -2	29	19	11

Mice were injected ip with 50 mg/kg PR 879-317A or iv with 500 mg/kg isoprinosine in 0.2 ml pyrogen-free saline 2 days or 4 days before test. Control mice received the same volume of pyrogen-free saline. This assay is based on the release of chromium from labeled YAC cells. Five mice per treatment group were examined. Percent cytotoxicity was calculated using the following formula:

$$100 \times \frac{\text{cpm release in test - spontaneous release}}{\text{maximum cpm release - spontaneous release}}$$

Table 34. Activation of splenic natural killer cells by rhuIFNα-B/D

Treatment	% Cytotoxicity Effector to target ratio		
	100:1	50:1	25:1
Control	13	9	8
rhuIFN i.v. 1×10^6 U	23*	14	10
rhuIFN i.p. 1×10^6 U	44**	32**	19**

* P<.05
**P<.001

Interferon was diluted in PBS on the day of administration and inoculated (0.2 ml containing indicated unit dose) one day prior to assay. This assay is based on the release of chromium from labeled YAC cells. Three mice per treatment group were examined. Percent cytotoxicity was calculated using the following formula:

$$100 \times \frac{\text{cpm release in test - spontaneous release}}{\text{maximum cpm release - spontaneous release}}$$

Table 35. Splenic B and T cell numbers in mice treated with recombinant interferon (r-IFN) or poly I:C-LC.

Treatment Group	Cells/Spleen ($\times 10^{-7}$)	B cells		T cells	
		%	Abs. No. ($\times 10^{-7}$)	%	Abs. No. ($\times 10^{-7}$)
Control	5.94	44	2.64	28	1.71
r-IFN (iv)	6.17	36*	2.15	25	1.45
r-IFN (ip)	6.76	42	2.85	22*	1.43
Poly I:C-LC	5.39	50	2.73	29	1.61

Mice were given recombinant interferon ($1x10^6$ IU), intravenously or intraperitoneally, or Poly I:C-LC (500 μg/kg), intravenously, one day before assaying for the numbers of splenic B and T cells. The numbers of B and T cells were determined using avidin-conjugated monoclonal anti-sIg or anti-Thy 1 antibodies, respectively, and FITC-labeled avidin. The percentage of B and T cells was determined using a Coulter Epics V flow cytometer and the Immuno data analysis program.

Abs. No. = Absolute numbers of B or T cells

* $p<0.05$

Table 36. Virus titers in brains and spinal cords of mice receiving intracranial administration of rhuIFNα-B/D for therapy of Banzi encephalitis.

Treatment	Virus Titers (per gram x 10^6)	
	Brain	Spinal Cord
rhuIFNα-B/D		
Day 0	0, 0, 0	0, 0, 0
Day 1	0, 173, 0	0, 13, 0
Day 2	865, 0, <1	45, 0, 0
Day 3	353, 295, <1	38, 29, 0
Day 4	17, 10, 2	2, 44, 0
Saline Control	34, 36, 2	8, <1, <1

Banzi virus (1 LD_{80}) was inoculated intraperitoneally to 6 week old male mice, and interferon administered i.c. (single dose 1 x 10^6 units) at the times indicated. Three mice per group were sacrificed 6 days after infection and their brains and spinal cords homogenized prior to plaque assay for infectious virus.

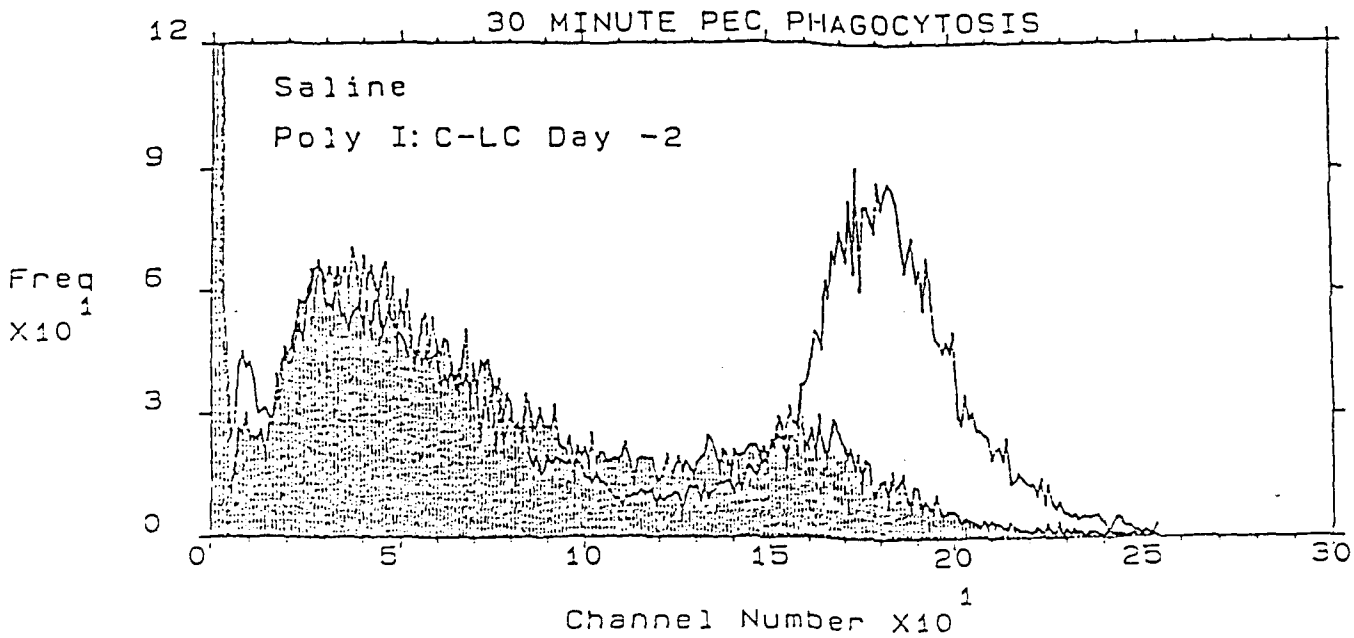


Figure 1. Flow Cytometric analysis of phagocytosis by peritoneal cells from mice treated with Poly I:C-LC (5 mg/kg) on day -2.

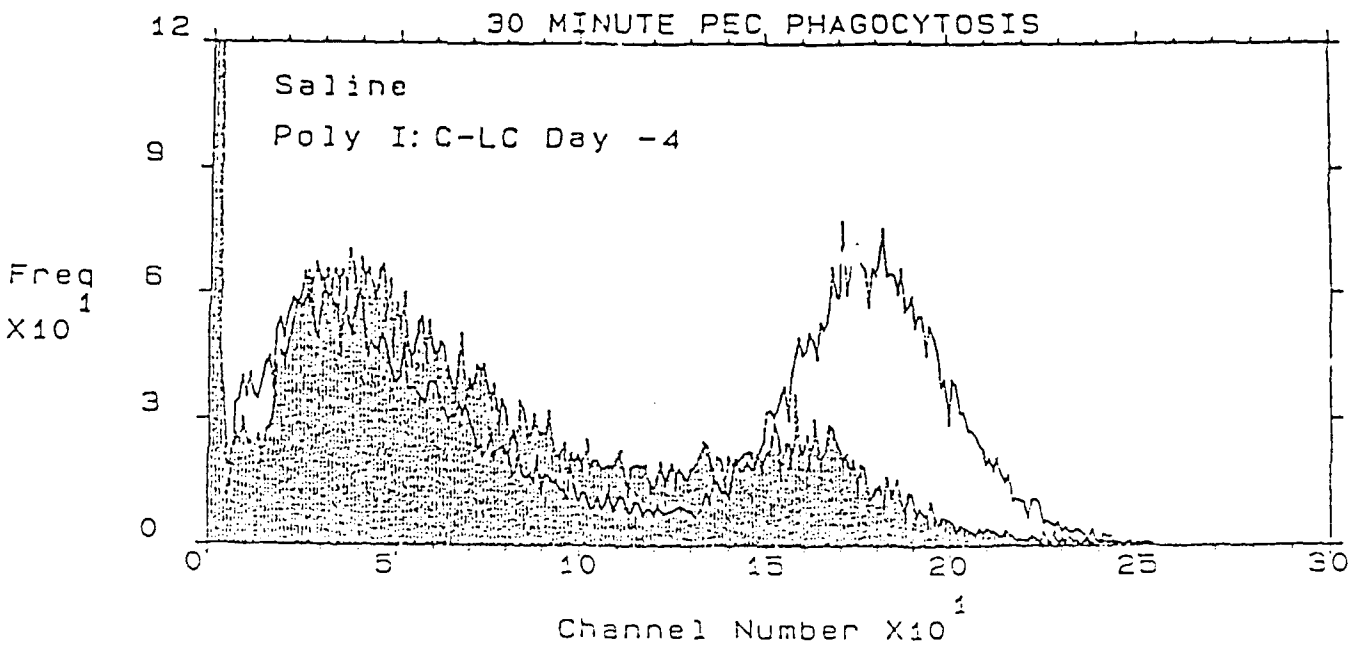


Figure 2. Flow Cytometric analysis of phagocytosis by peritoneal cells from mice treated with Poly I:C-LC (5 mg/kg) on day -4.

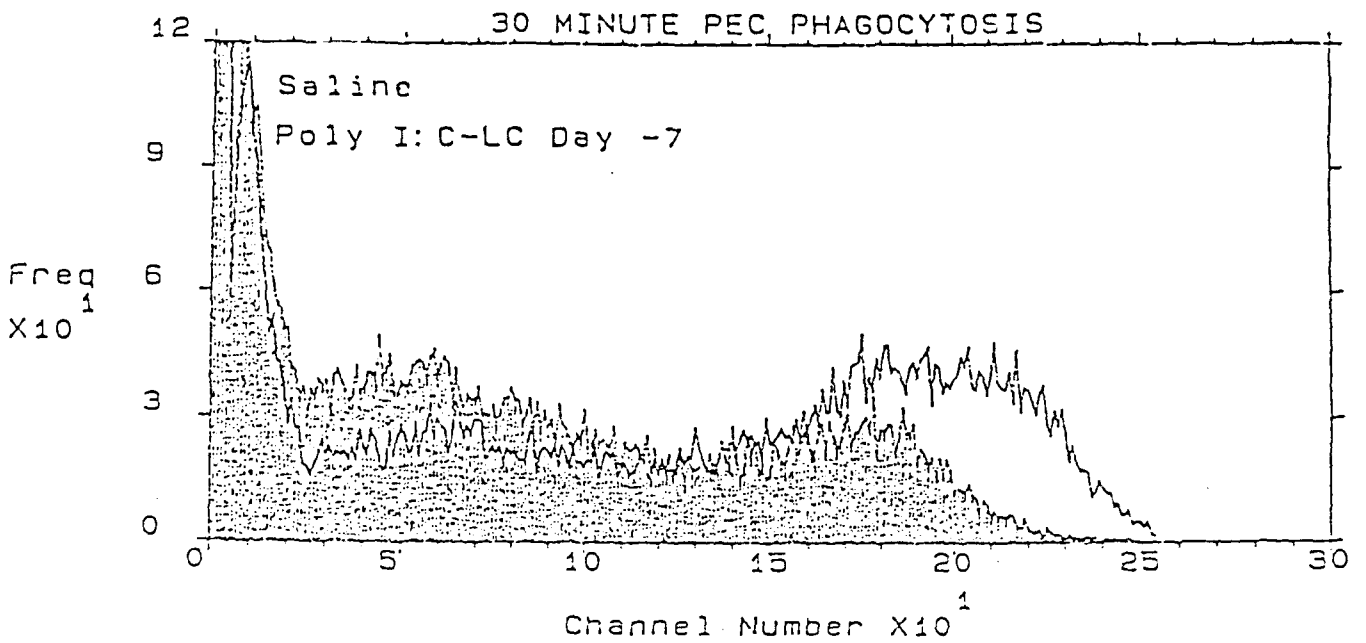


Figure 3. Flow Cytometric analysis of phagocytosis by peritoneal cells from mice treated with Poly I:C-LC (5 mg/kg) on day -7.

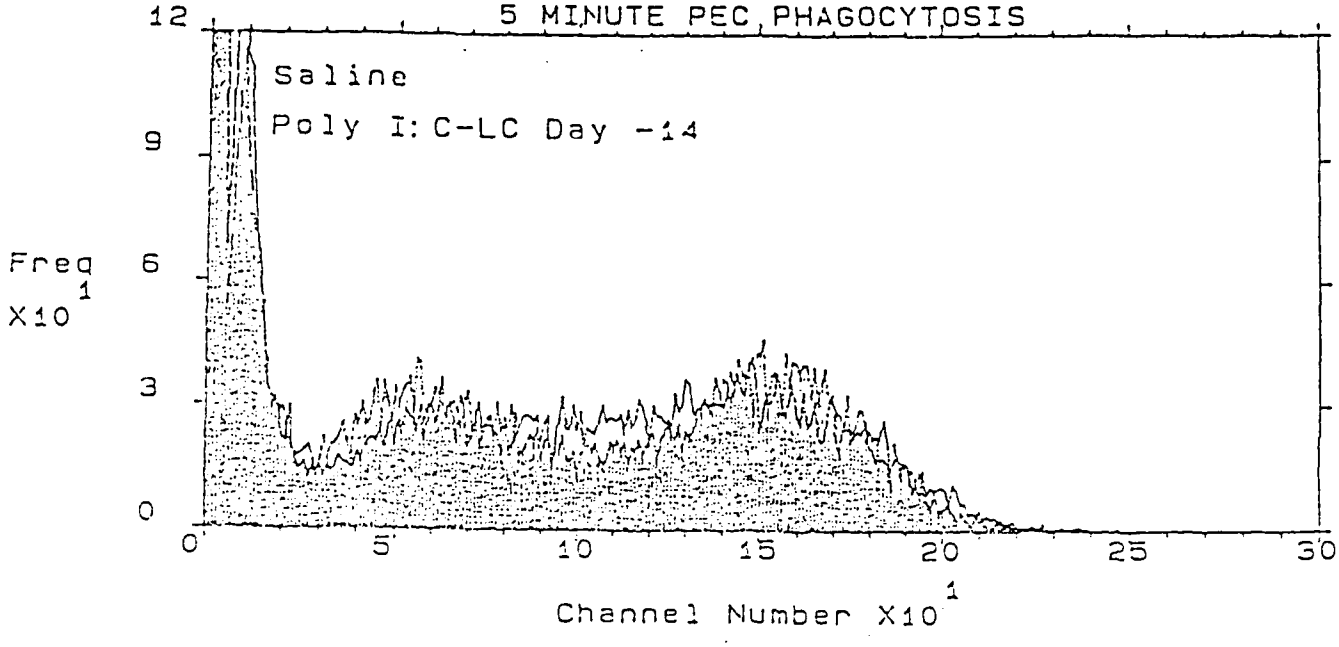


Figure 4. Flow Cytometric analysis of phagocytosis by peritoneal cells from mice treated with Poly I:C-LC (5 mg/kg) on day -14.

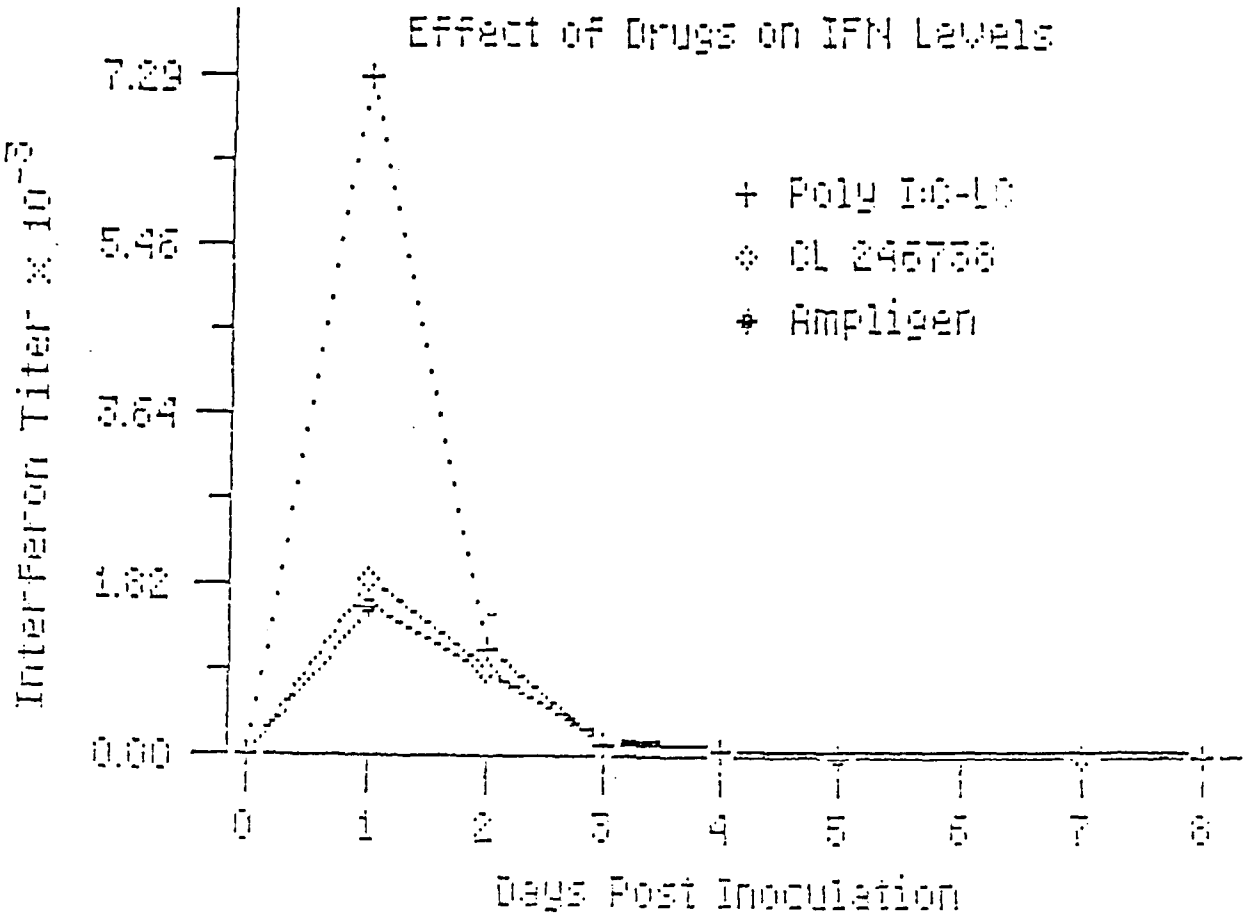


Figure 5. Serum interferon levels at various times after drug treatment. Poly I:C-LC and Ampligen (5 mg/kg) were given iv and CL 246738 (200 mg/kg) was given orally. Each point represents the mean of three mice.

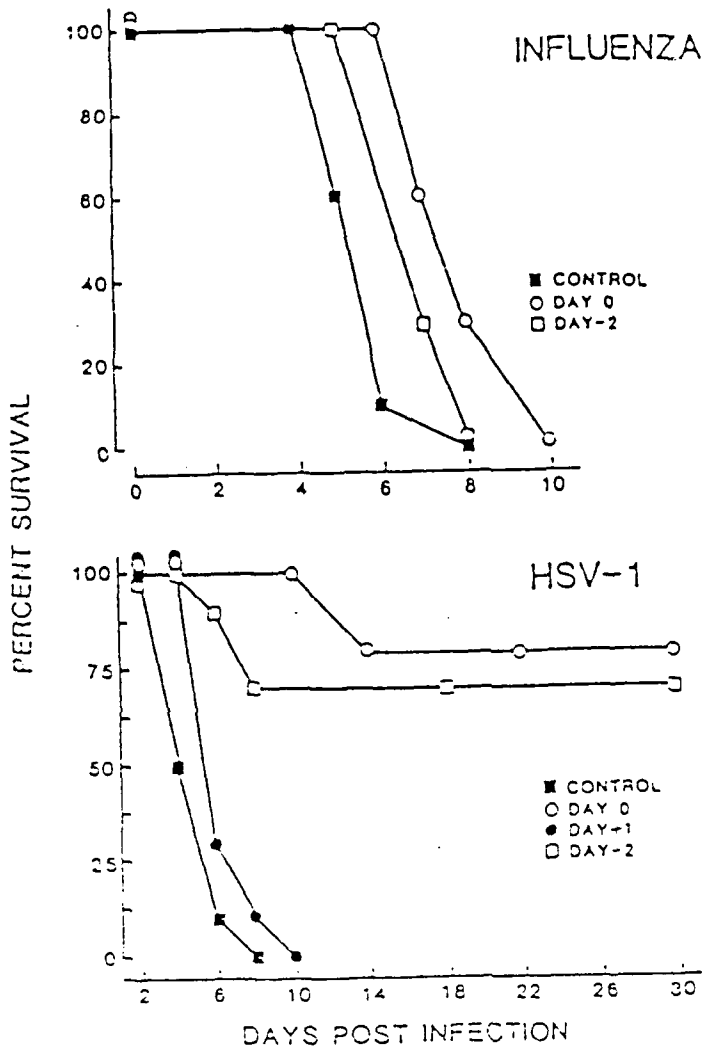


Figure 6. Effect of iv injection of Poly I:C-LC (5 mg/kg) on resistance to Influenza or HSV-1 induced pneumonitis. Drug was administered on the days indicated.

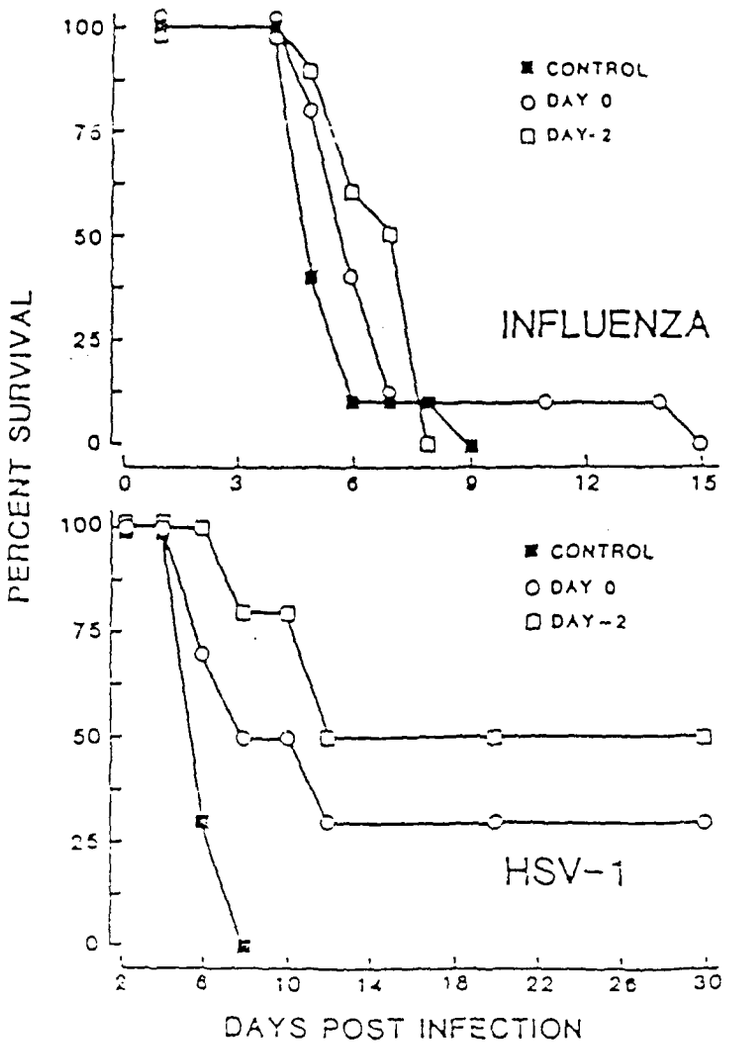


Figure 7. Effect of oral administration of CL 246738 (200 mg/kg) on resistance to Influenza or HSV-1 induced pneumonitis. Drug was administered on the days indicated.

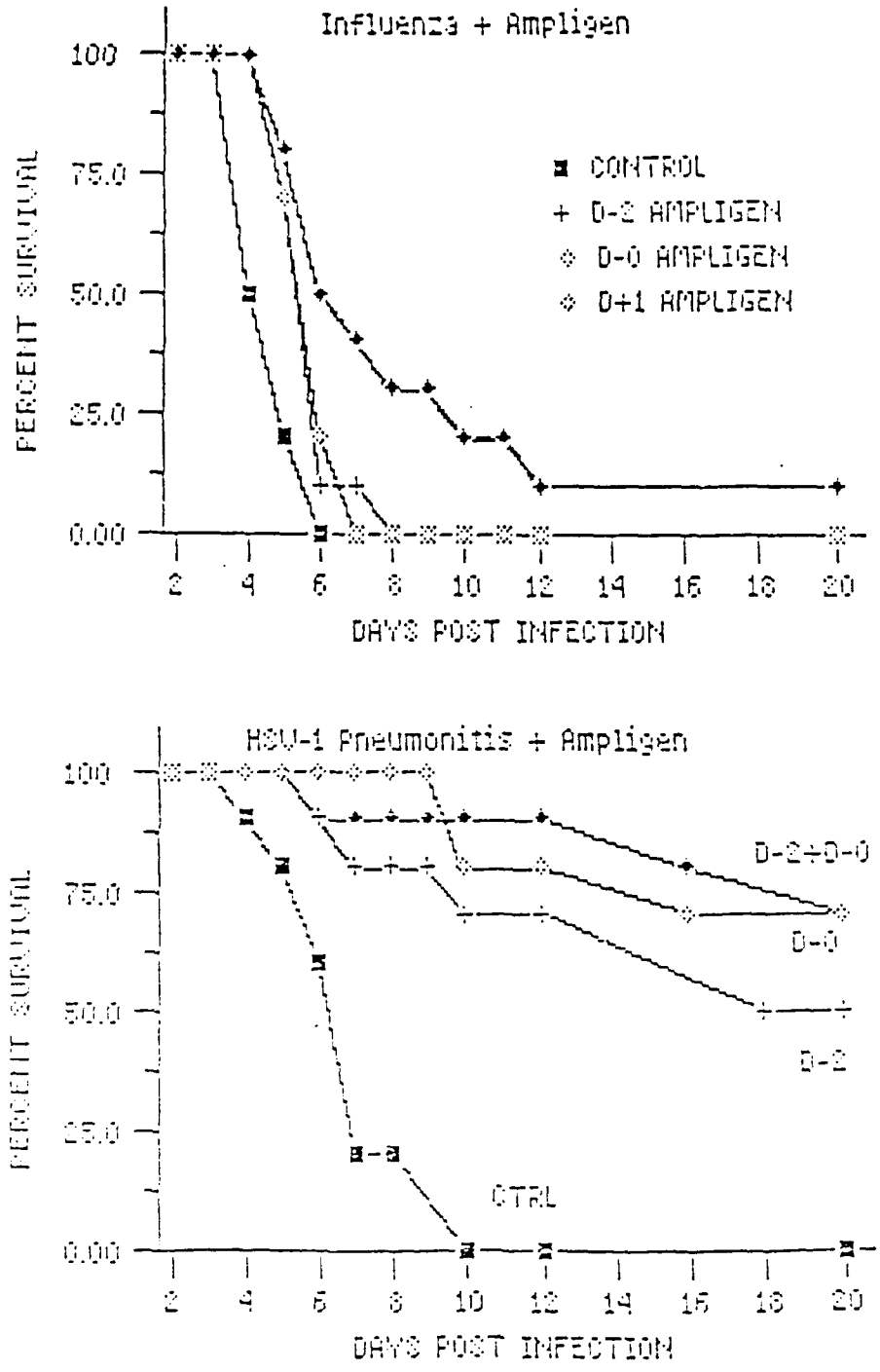


Figure 8. Effect of iv injection of Ampligen (5 mg/kg) on resistance to Influenza or HSV-1 induced pneumonitis. Drug was administered on the days indicated.

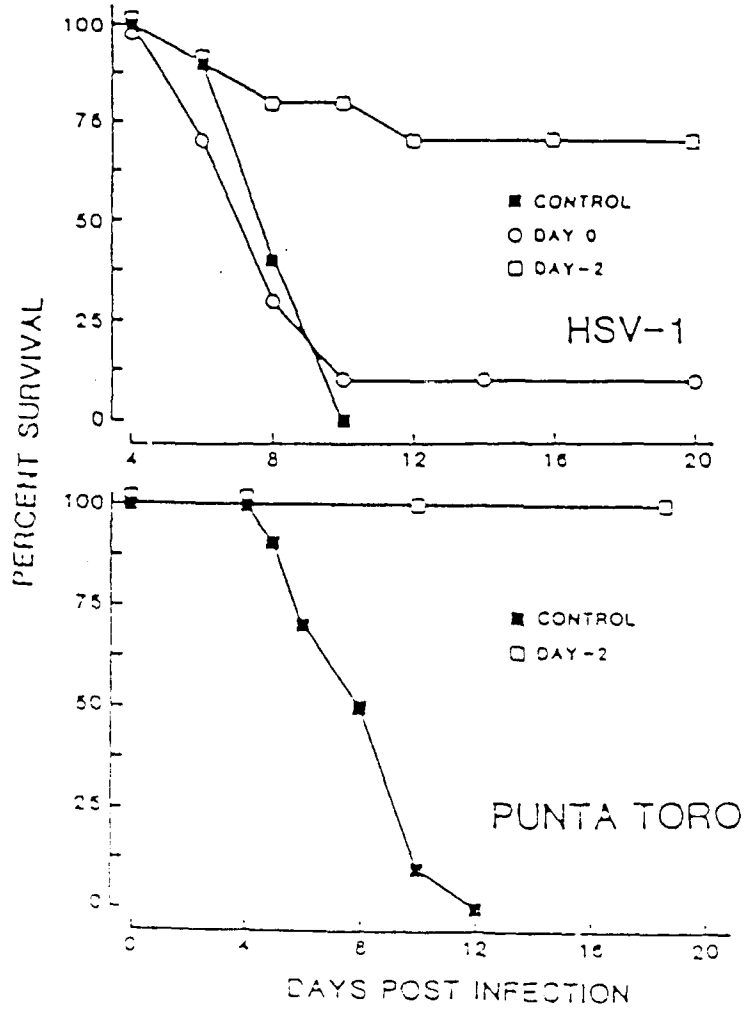


Figure 9. Effect of iv injection of Poly I:C-LC (5 mg/kg) on resistance to HSV-1 or Punta Toro induced hepatitis. Drug was administered on the days indicated.

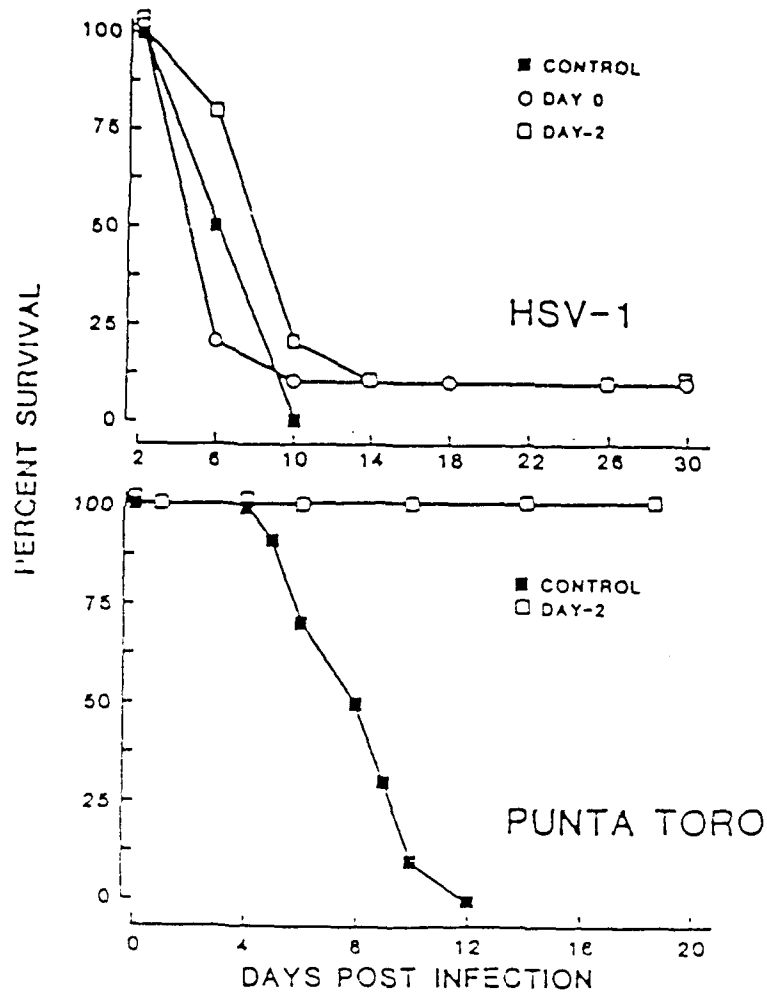


Figure 10. Effect of oral administration of CL 246738 (200 mg/kg) on resistance to HSV-1 or Punta Toro induced hepatitis. Drug was administered on the days indicated.

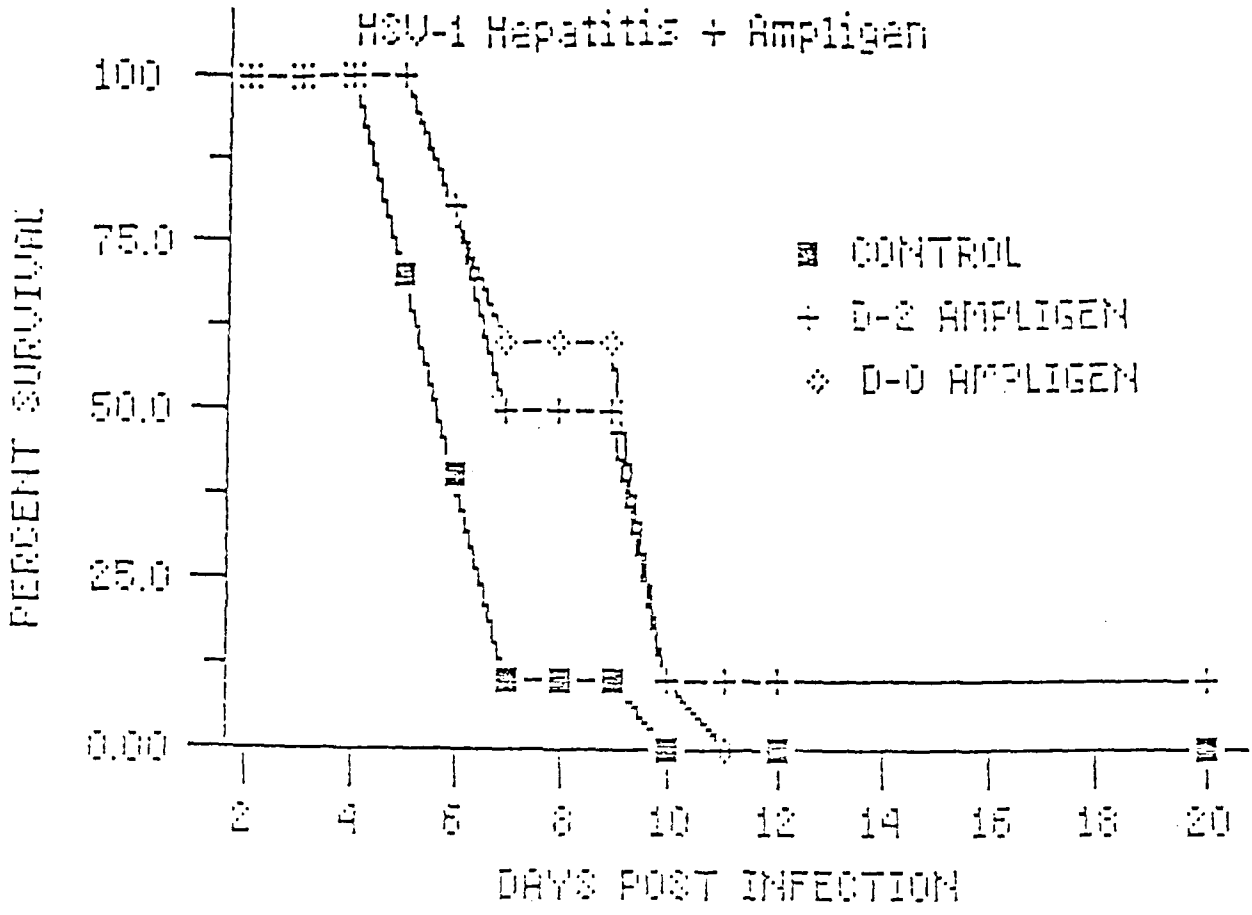


Figure 11. Effect of iv injection of Ampligen (5 mg/kg) on resistance to HSV-1 induced hepatitis. Drug was administered on the days indicated.

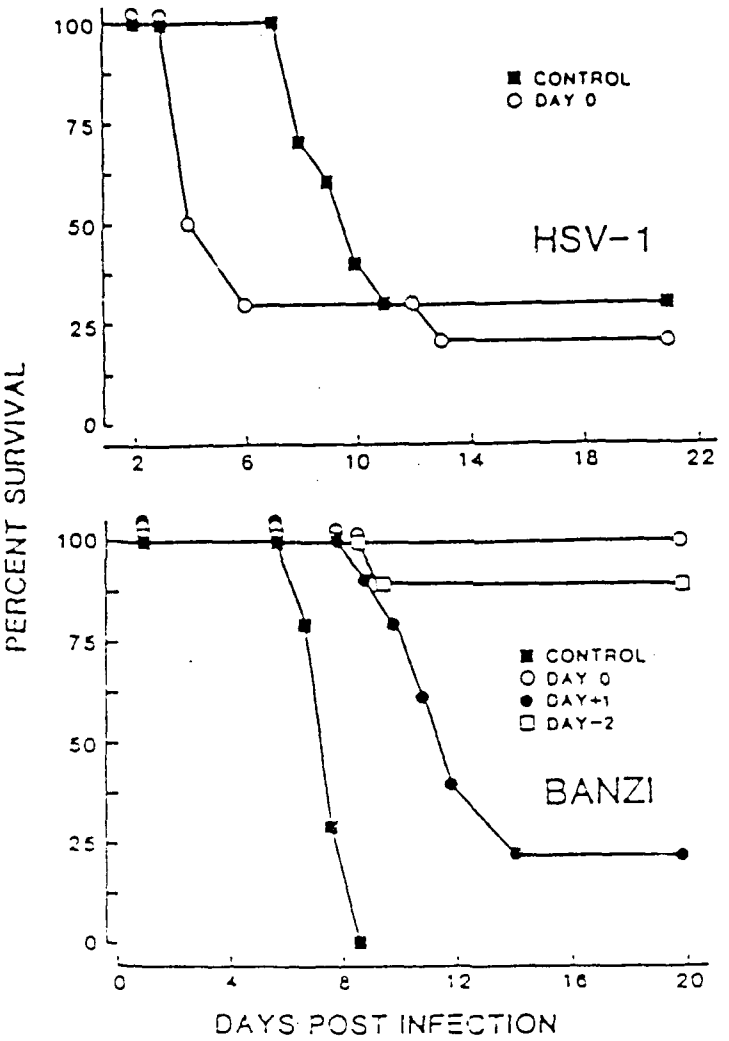


Figure 12. Effect of iv injection of Poly I:C-LC (5 mg/kg) on resistance to HSV-1 or Banzi virus induced encephalitis. Drug was administered on the days indicated.

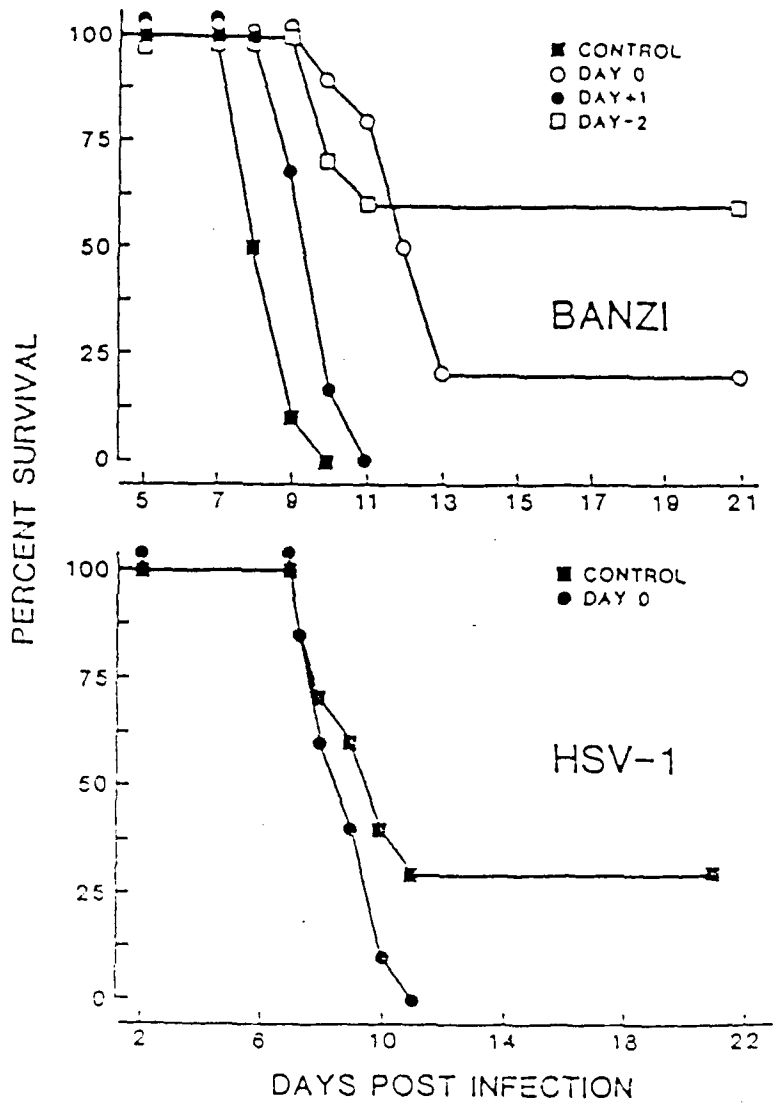


Figure 13. Effect of oral administration of CL 246738 (200 mg/kg) on resistance to HSV-1 or Banzi virus induced encephalitis. Drug was administered on the days indicated.

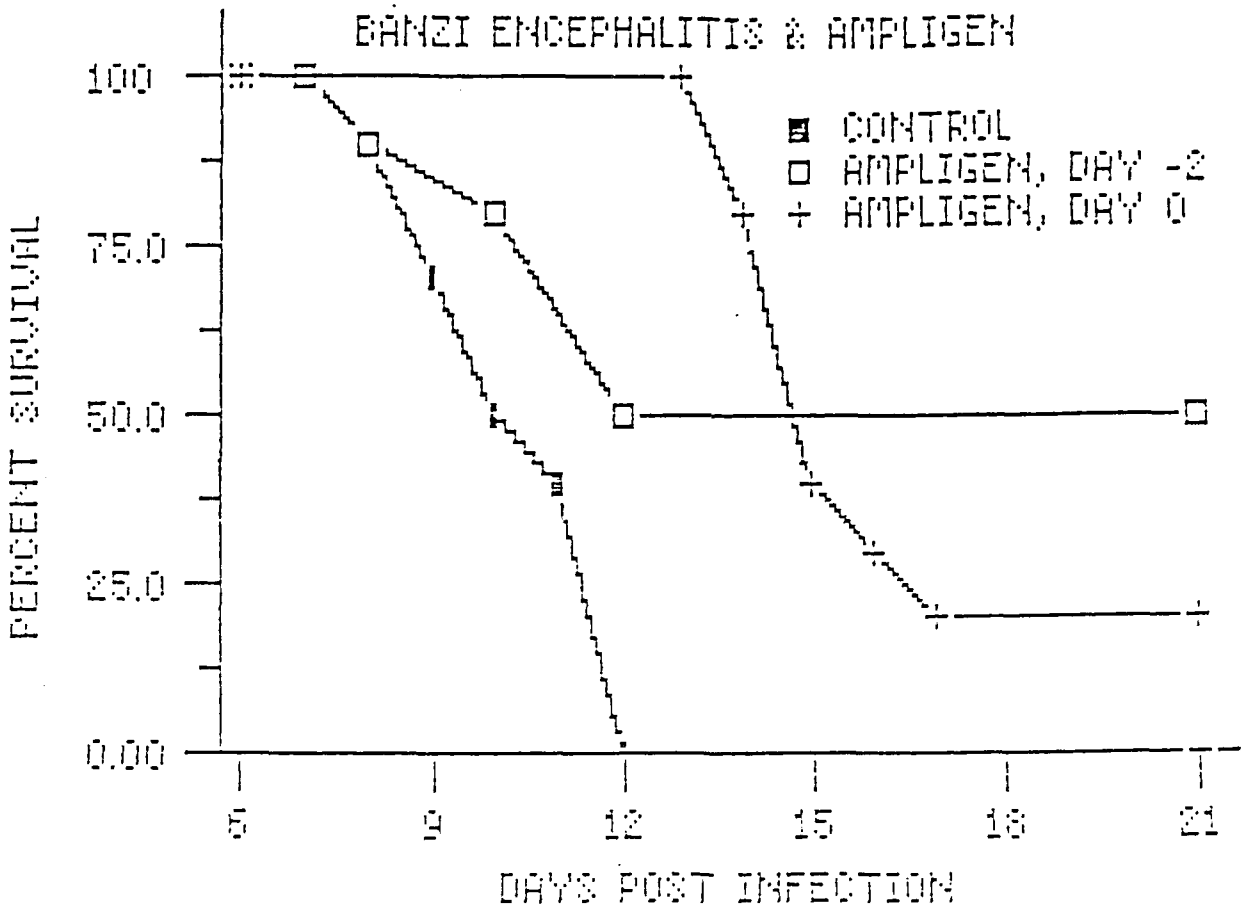


Figure 14. Effect of iv injection of Ampligen (5 mg/kg) on resistance to Banzi virus induced encephalitis. Drug was administered on the days indicated.

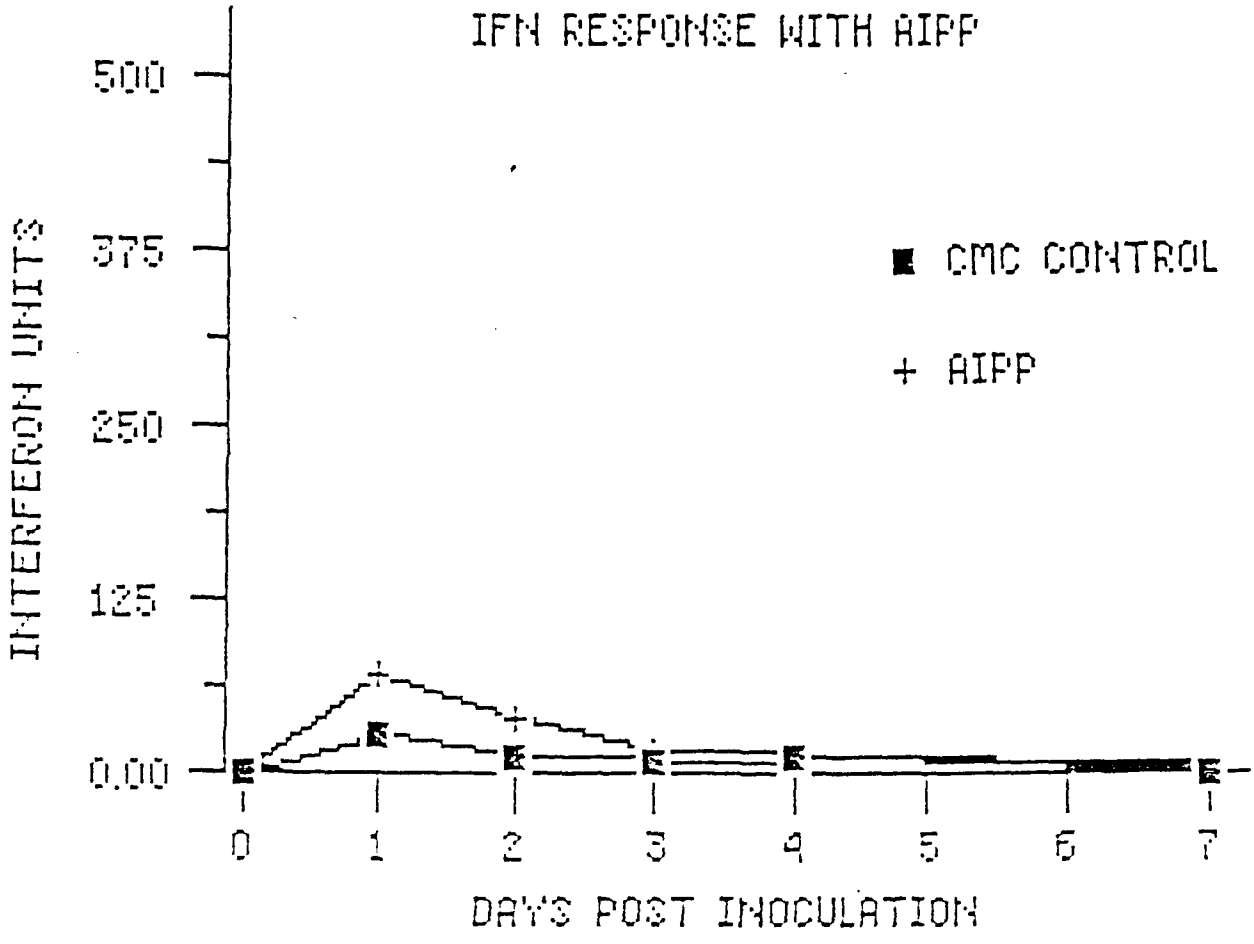


Figure 15. Serum interferon levels following treatment with AIPP. Mice were injected ip with 0.2 ml CMC or 250 mg/kg drug in 0.2 ml CMC and then bled on days indicated. Day 0 bleed was obtained immediately before injection. Each point represents a mean of three mice.

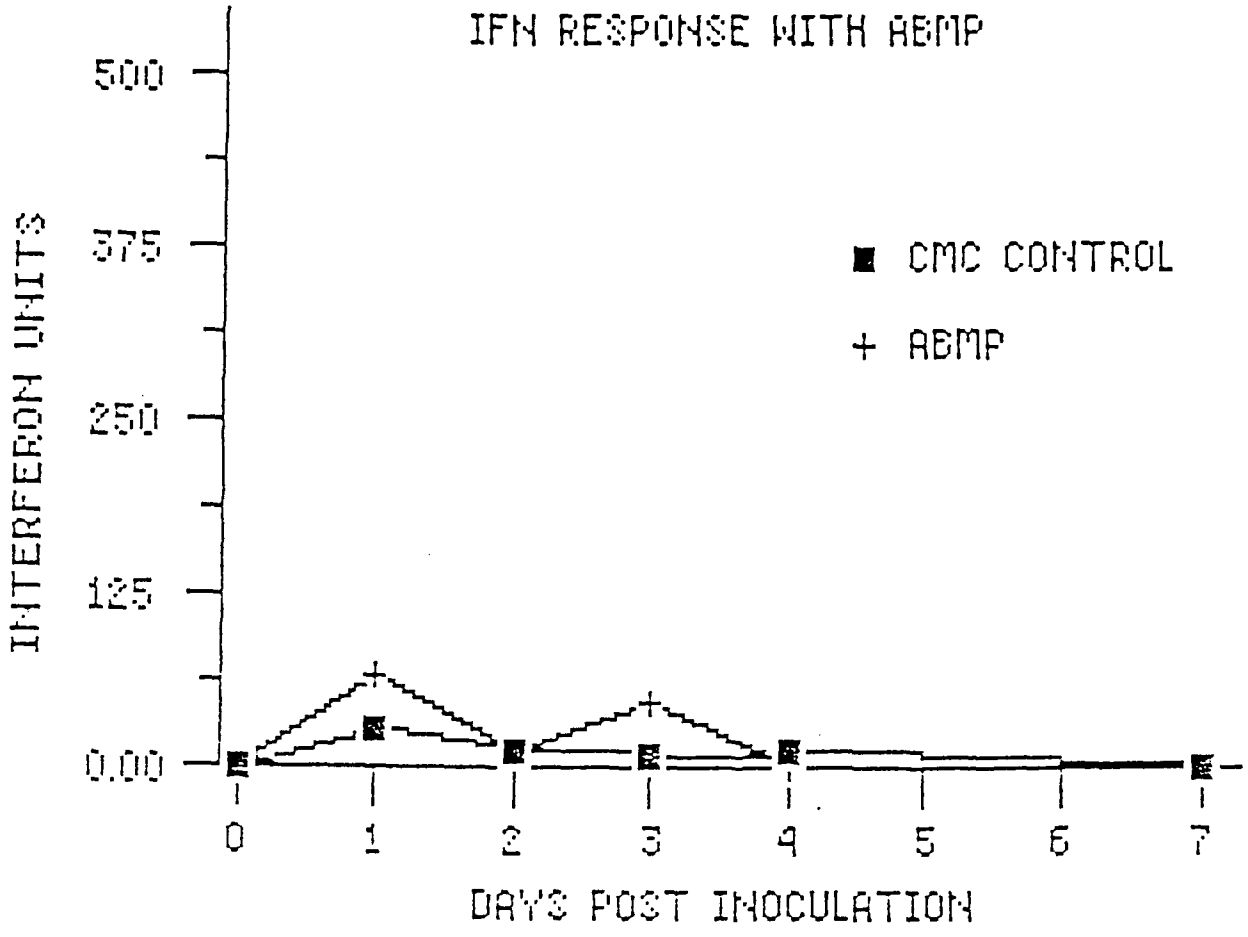


Figure 16. Serum interferon levels following treatment with ABMP. Mice were injected ip with 0.2 ml CMC or 250 mg/kg drug in 0.2 ml CMC and then bled on days indicated. Day 0 bleed was obtained immediately before injection. Each point represents a mean of three mice.

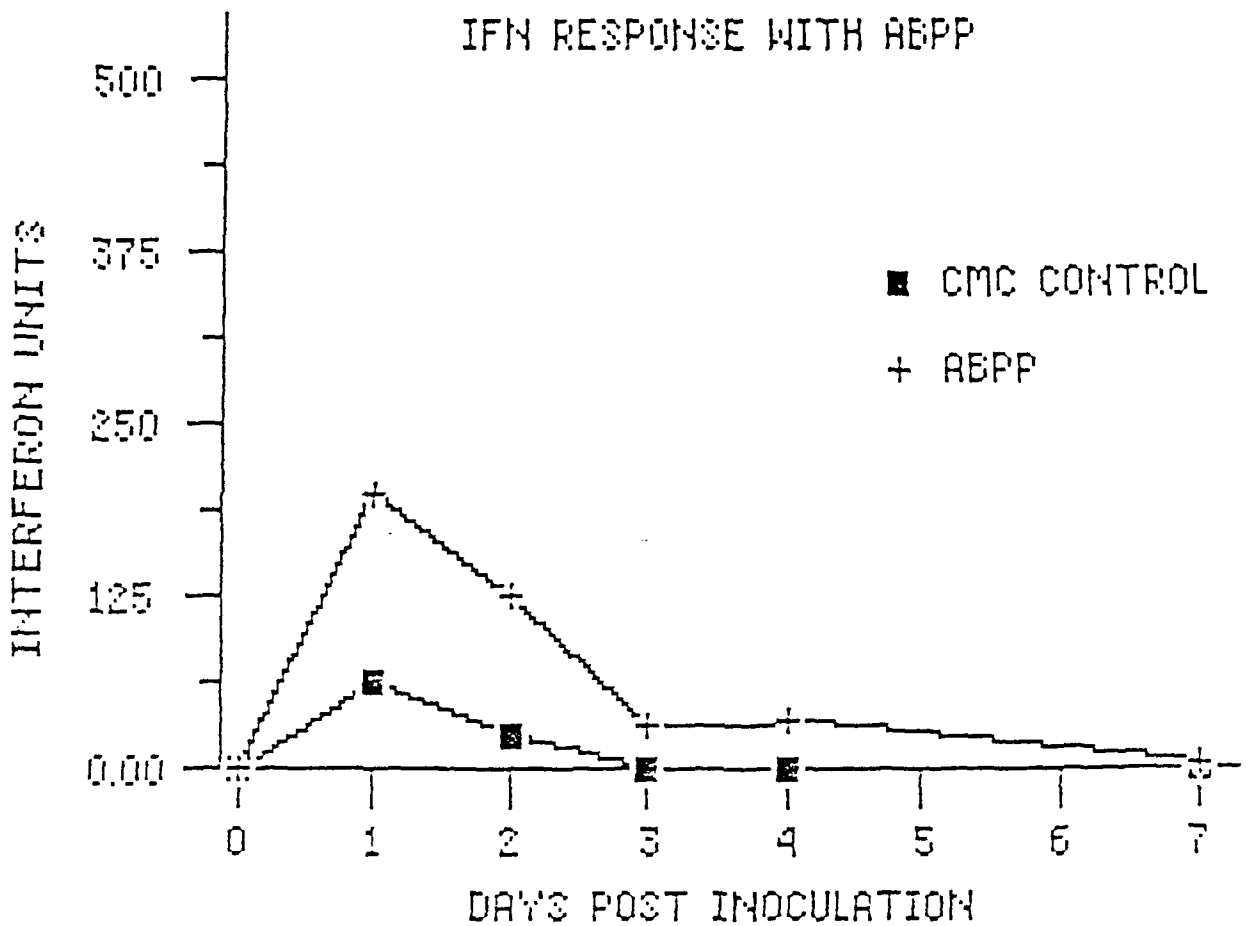


Figure 17. Serum interferon levels following treatment with ABPP. Mice were injected ip with 0.2 ml CMC or 250 mg/kg drug in 0.2 ml CMC and then bled on days indicated. Day 0 bleed was obtained immediately before injection. Each point represents a mean of three mice.

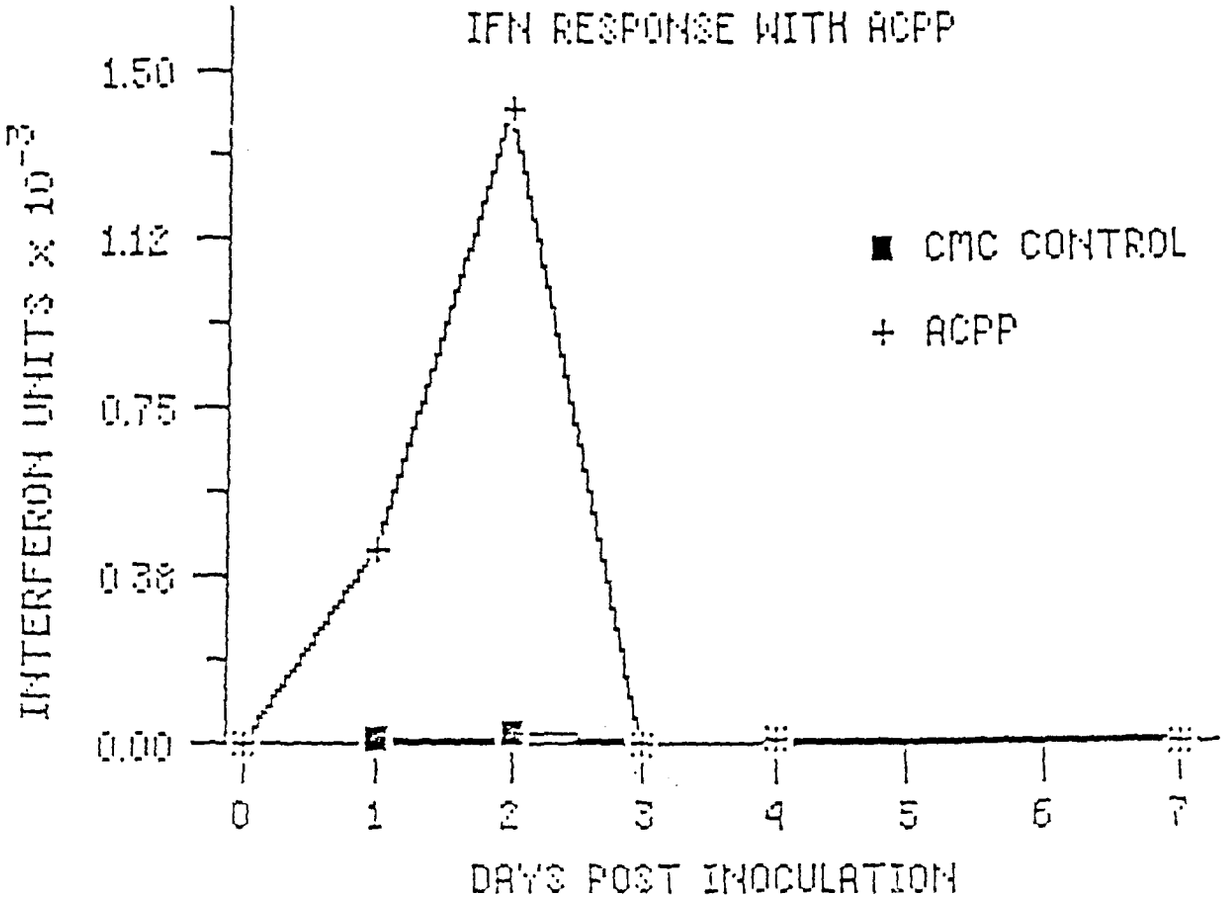


Figure 18. Serum interferon levels following treatment with ACPP. Mice were injected ip with 0.2 ml CMC or 250 mg/kg drug in 0.2 ml CMC and then bled on days indicated. Day 0 bleed was obtained immediately before injection. Each point represents a mean of three mice.

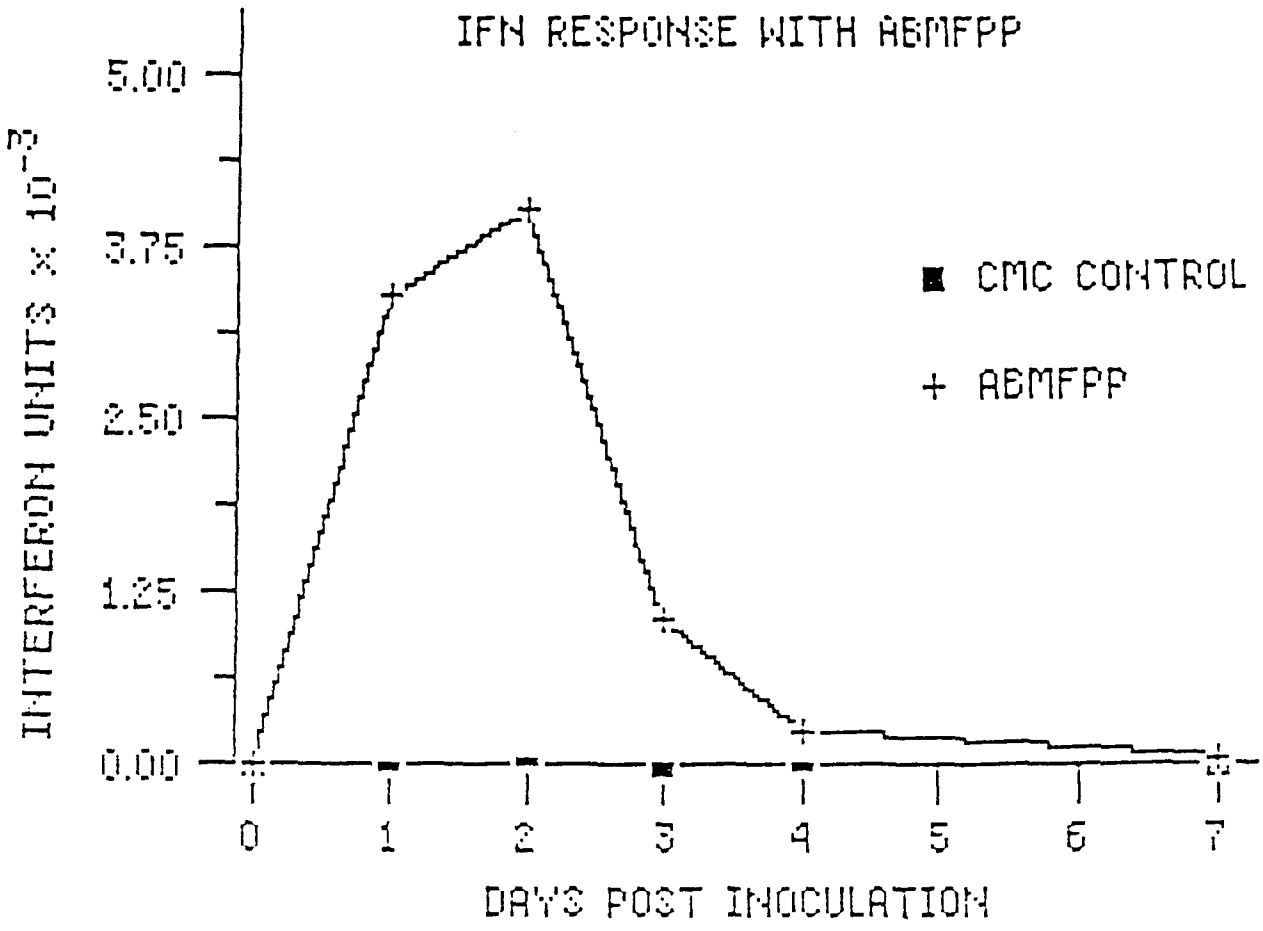
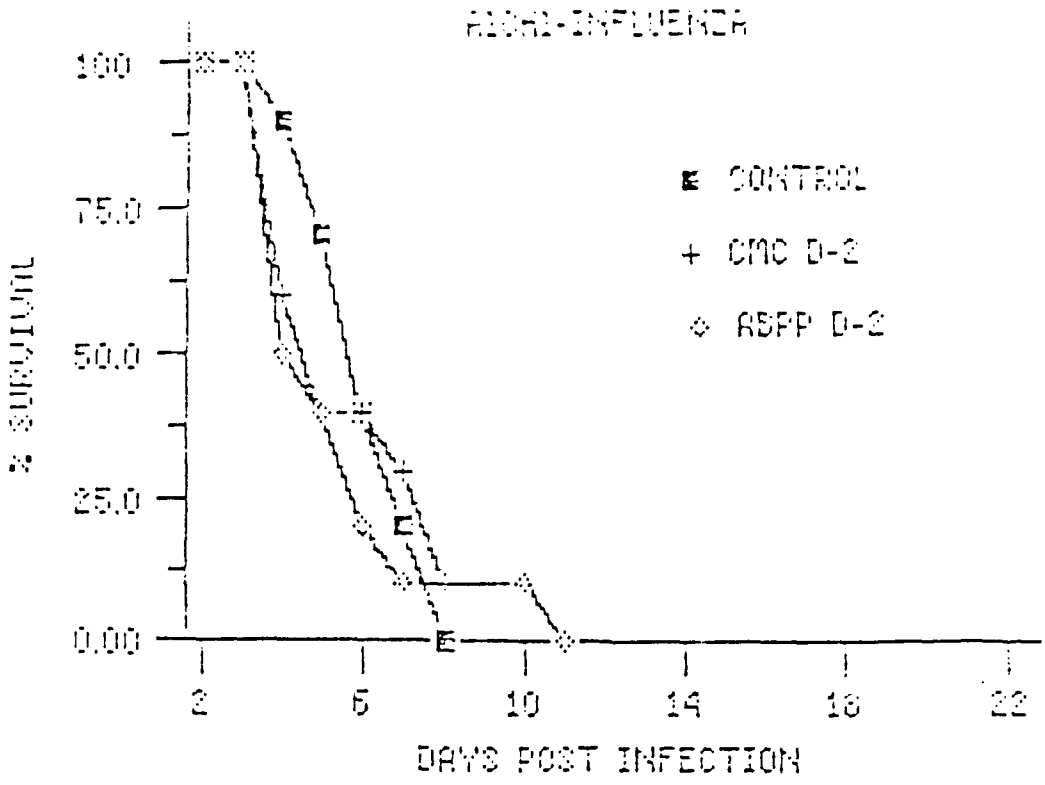


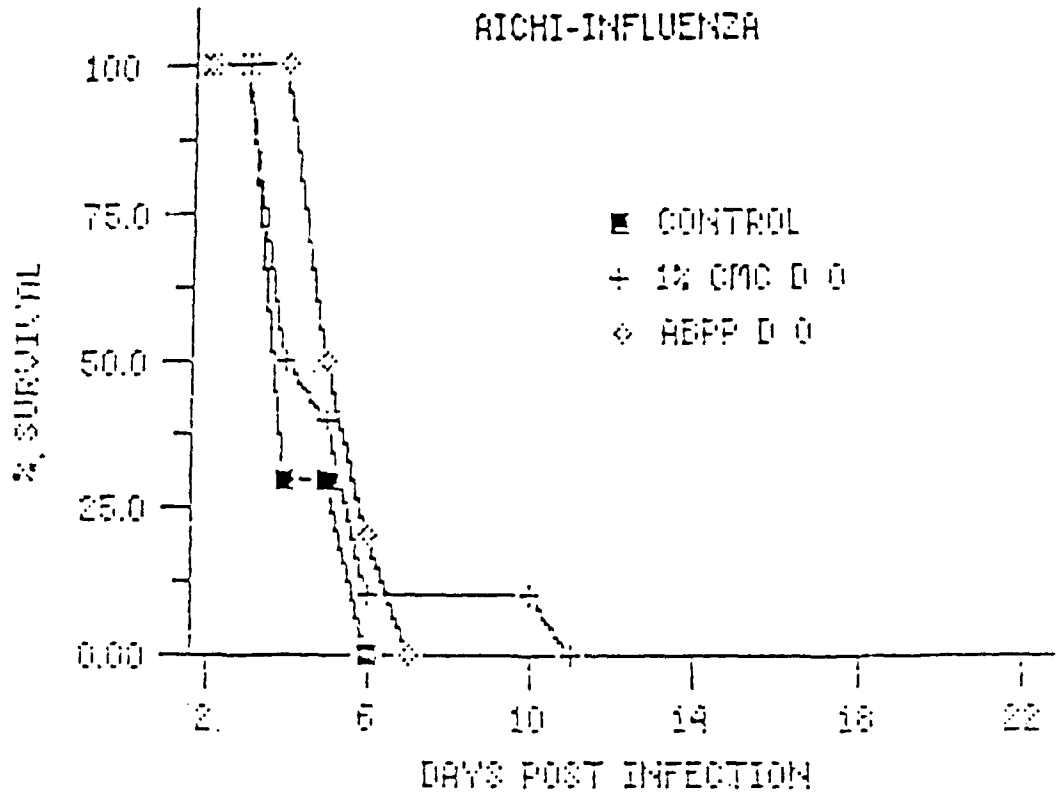
Figure 19. Serum interferon levels following treatment with ABMFPP. Mice were injected ip with 0.2 ml CMC or 250 mg/kg drug in 0.2 ml CMC and then bled on days indicated. Day 0 bleed was obtained immediately before injection. Each point represents a mean of three mice.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.96	-
ABPP Day -2	5.21	NS
Saline Control	6.07	NS

Figure 20. Effect of ABPP, given on day -2, on resistance to influenza-induced pneumonitis.

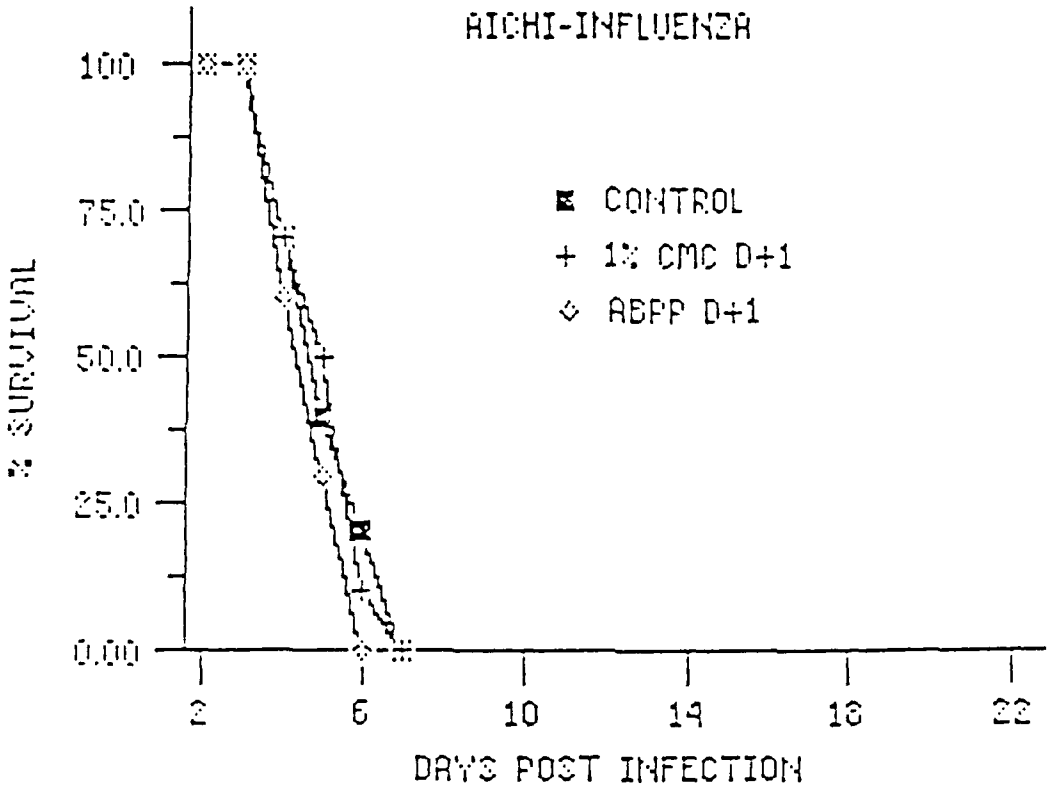
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.11	-
ABPP Day 0	5.65	NS
Saline Control	4.52	NS

Figure 21. Effect of ABPP, given on day 0, on resistance to influenza-induced pneumonitis.

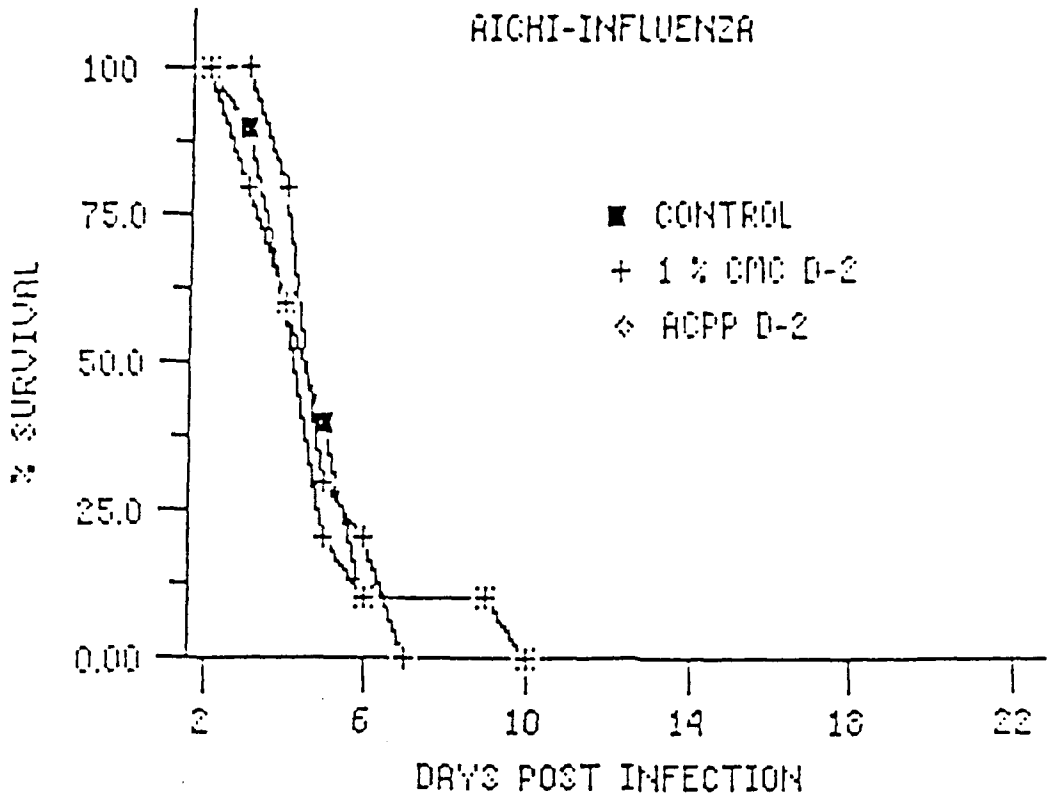
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.20	-
ABPP Day +1	4.83	NS
Saline Control	5.19	NS

Figure 22. Effect of ABPP, given on day +1, on resistance to influenza-induced pneumonitis.

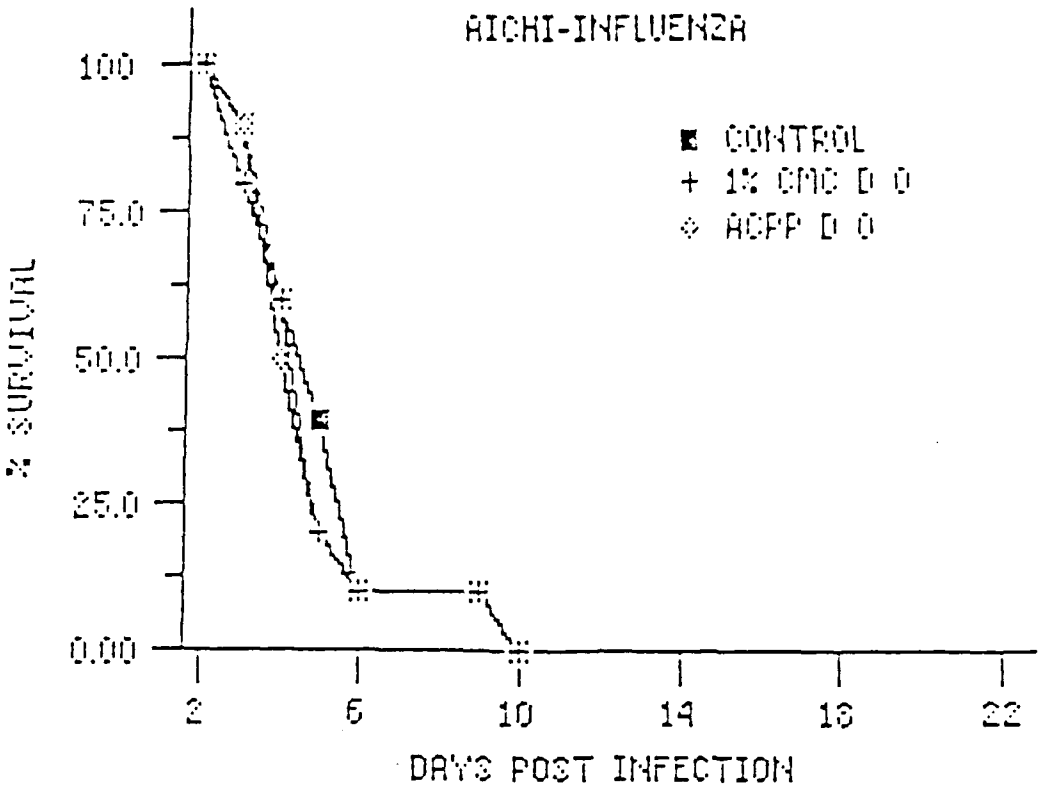
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.21	-
ACPP Day -2	5.21	NS
Saline Control	4.80	NS

Figure 23. Effect of ACPP, given on day -2, on resistance to influenza-induced pneumonitis.

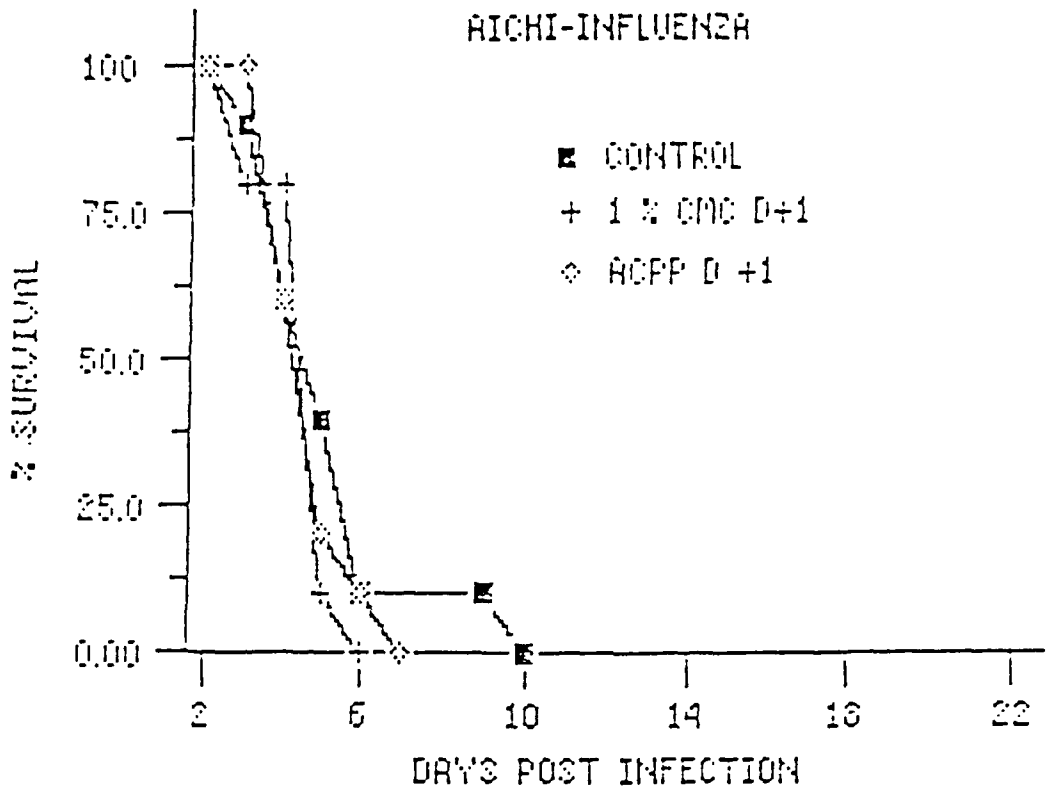
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	4.61	-
ACPP Day 0	4.74	NS
Saline Control	4.80	NS

Figure 24. Effect of ACPP, given on day 0, on resistance to influenza-induced pneumonitis.

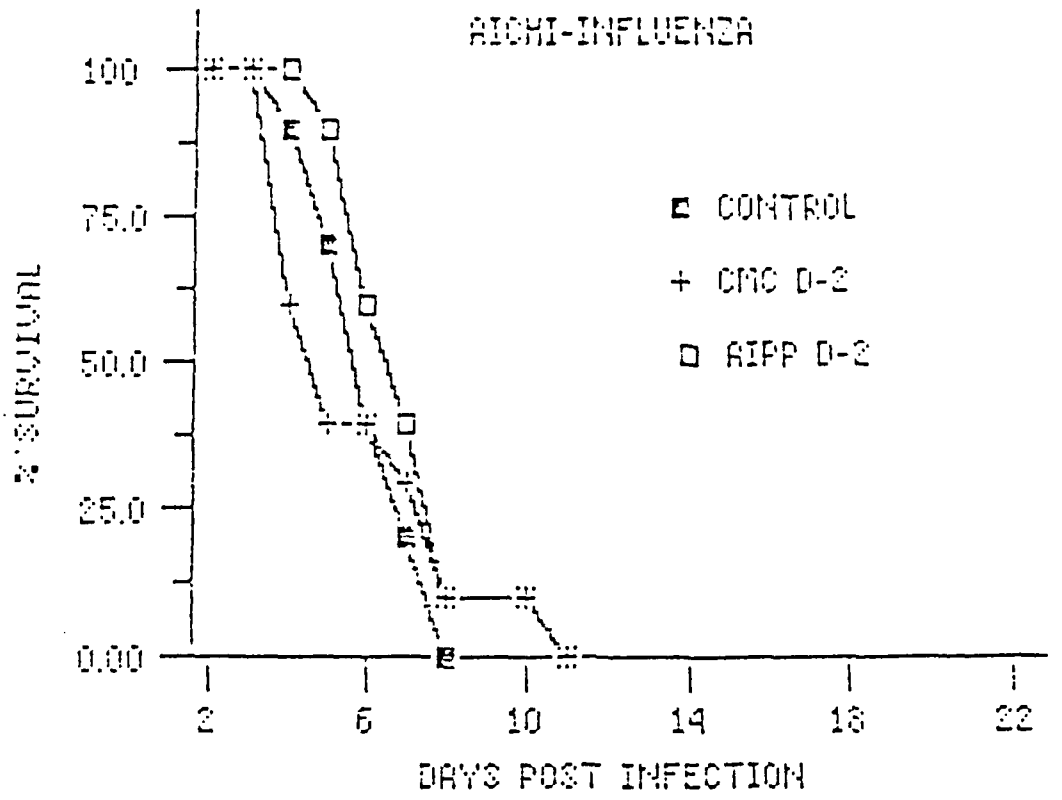
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	4.60	-
ACPP Day +1	4.82	NS
Saline Control	4.80	NS

Figure 25. Effect of ACPP, given on day +1, on resistance to influenza-induced pneumonitis.

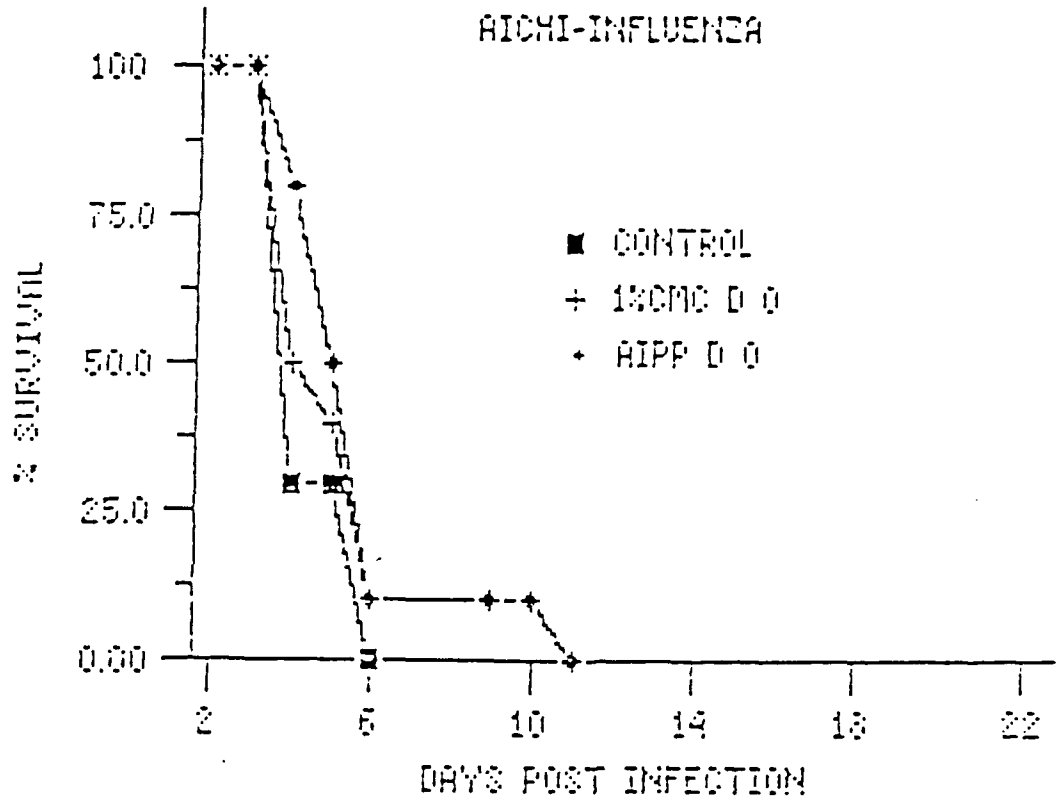
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.96	-
AIPP Day -2	7.04	NS
Saline Control	6.07	NS

Figure 26. Effect of AIPP, given on day -2, on resistance to influenza-induced pneumonitis.

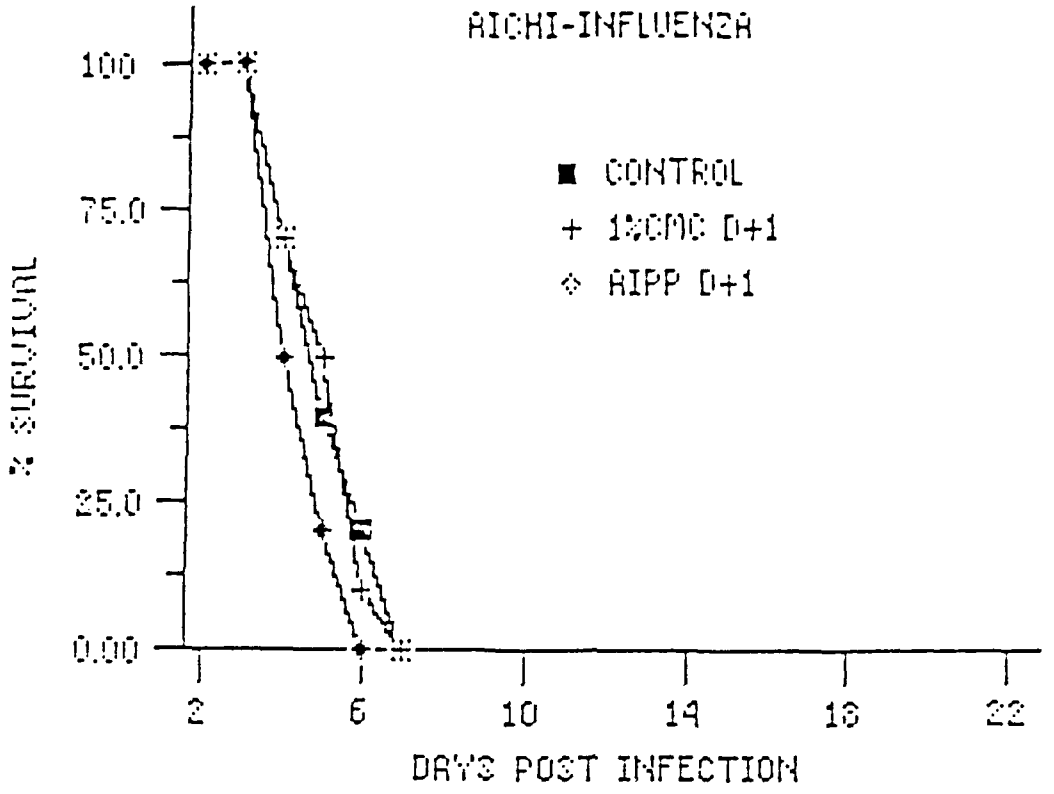
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.11	-
AIPP Day 0	5.57	NS
Saline Control	4.52	NS

Figure 27. Effect of AIPP, given on day 0, on resistance to influenza-induced pneumonitis.

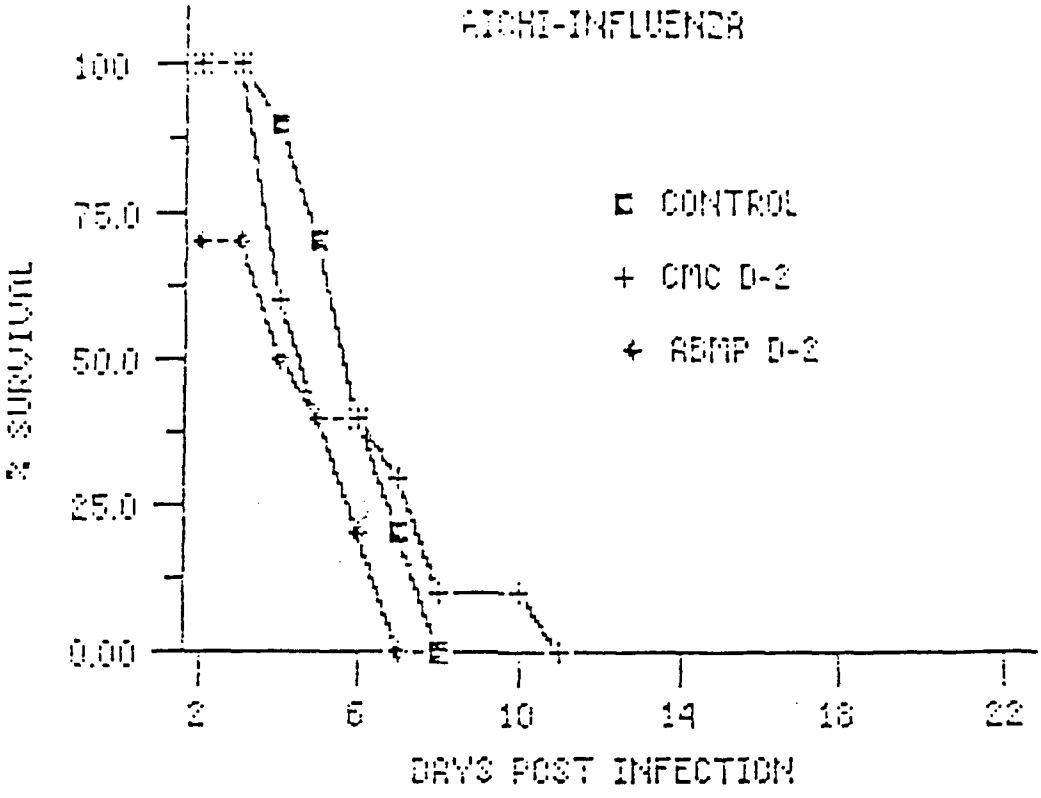
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.20	-
AIPP Day +1	4.87	NS
Saline Control	5.19	NS

Figure 28. Effect of AIPP, given on day +1, on resistance to influenza-induced pneumonitis.

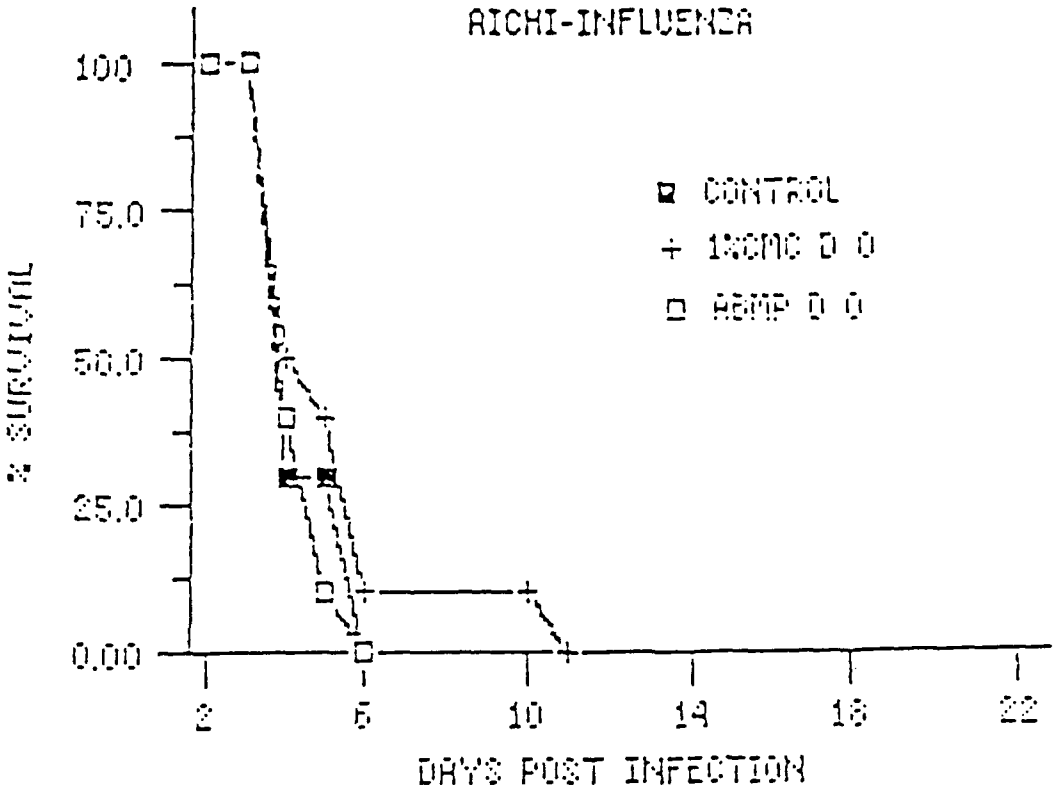
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.96	-
ABMP Day -2	5.38	NS
Saline Control	6.07	NS

Figure 29. Effect of ABMP, given on day -2, on resistance to influenza-induced pneumonitis.

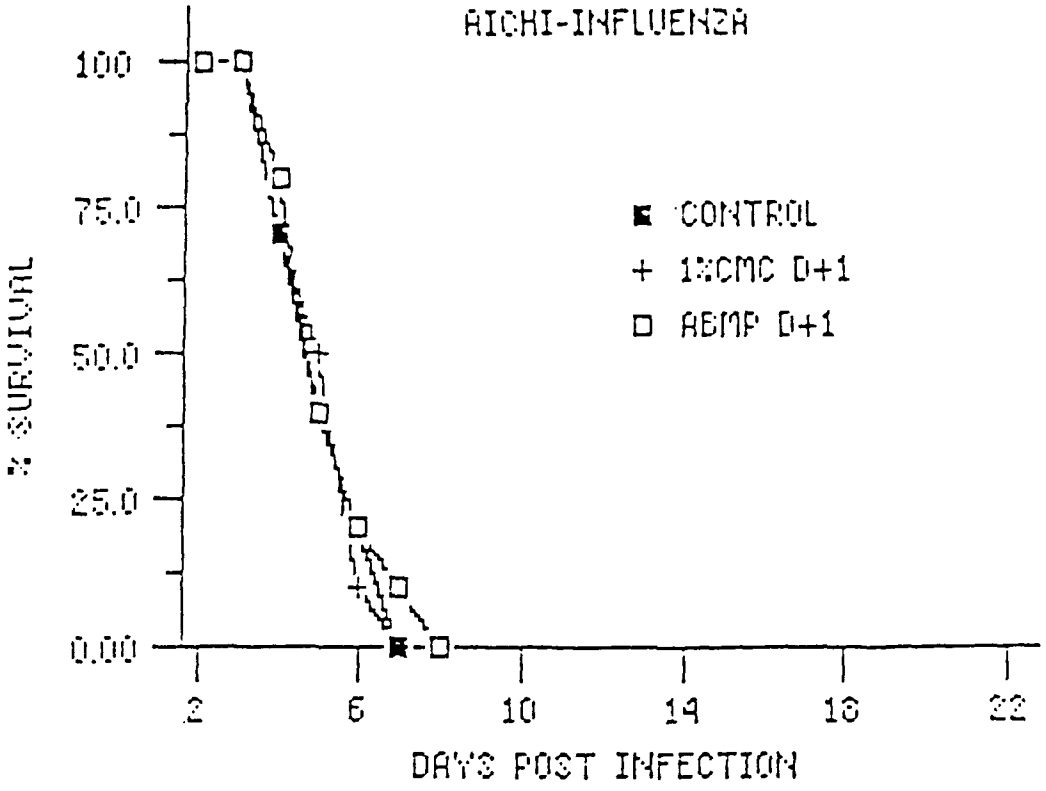
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.11	-
ABMP Day 0	4.45	NS
Saline Control	4.52	NS

Figure 30. Effect of ABMP, given on day 0, on resistance to influenza-induced pneumonitis.

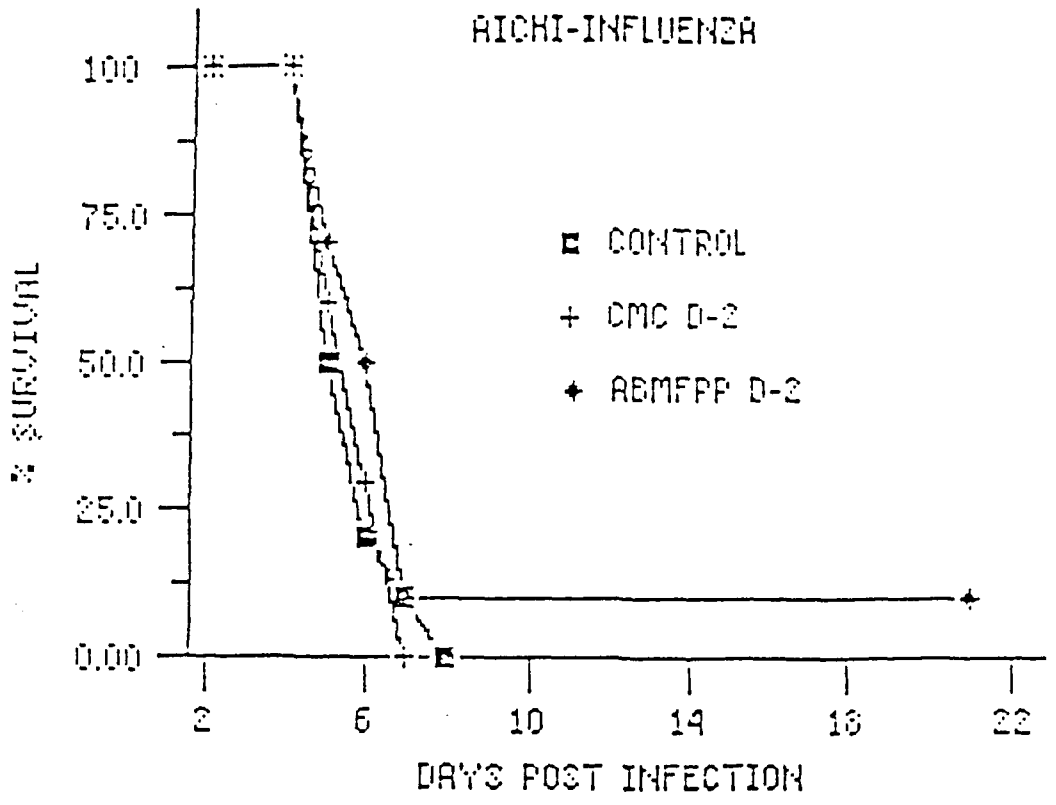
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.20	-
ABMP Day +1	5.38	NS
Saline Control	5.19	NS

Figure 31. Effect of ABMP, given on day +1, on resistance to influenza-induced pneumonitis.

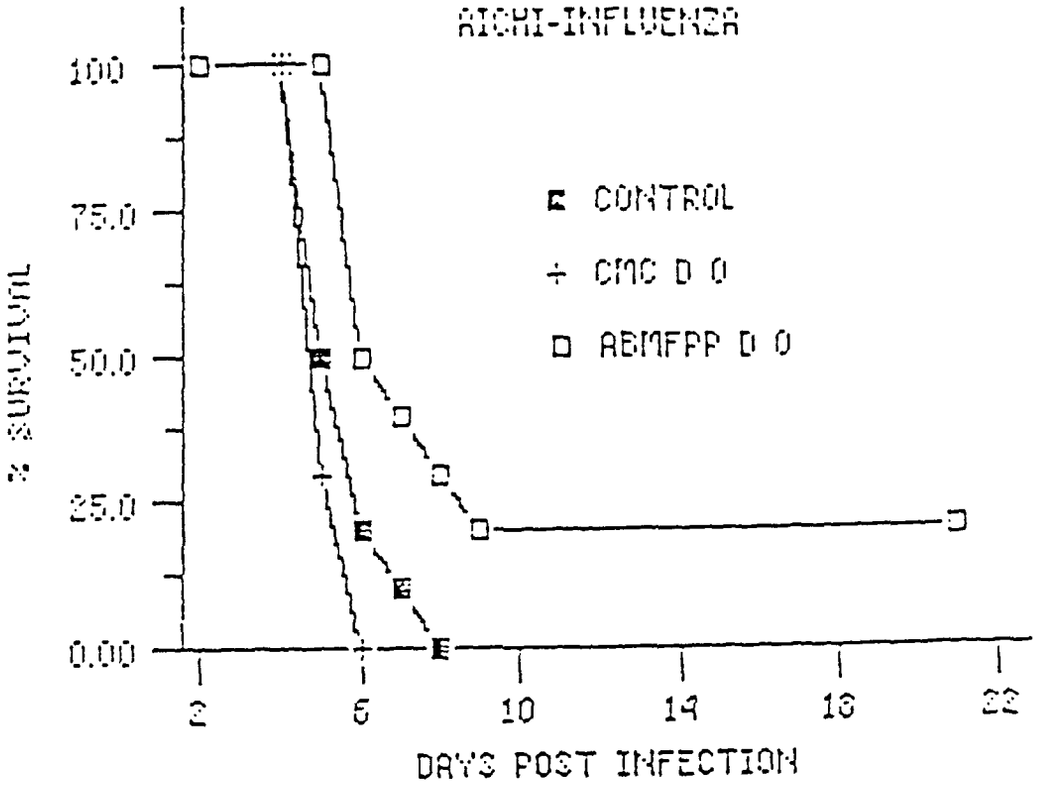
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.84	-
ABMFPP Day -2	6.85	NS
Saline Control	5.72	NS

Figure 32. Effect of ABMFPP, given on day -2, on resistance to influenza-induced pneumonitis.

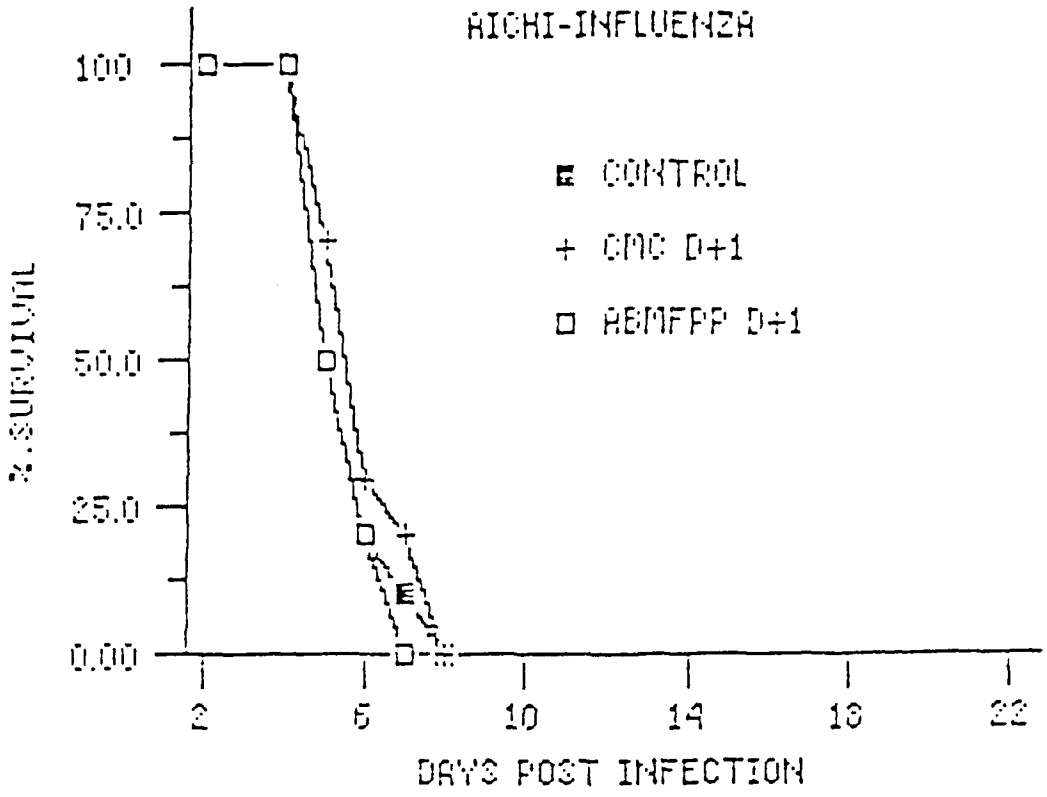
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.28	-
ABMFPP Day 0	8.39	<0.02
Saline Control	5.72	NS

Figure 33. Effect of ABMFPP, given on day 0, on resistance to influenza-induced pneumonitis.

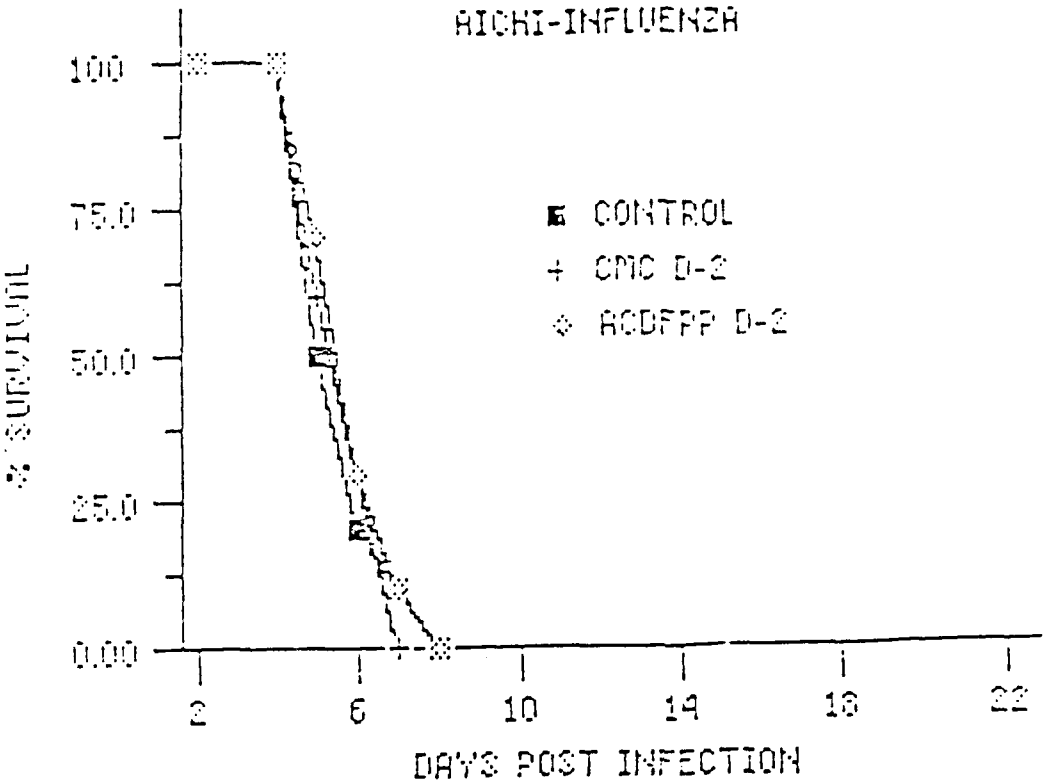
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.11	-
ABMFPP Day +1	5.65	NS
Saline Control	5.72	NS

Figure 34. Effect of ABMFPP, given on day +1, on resistance to influenza-induced pneumonitis.

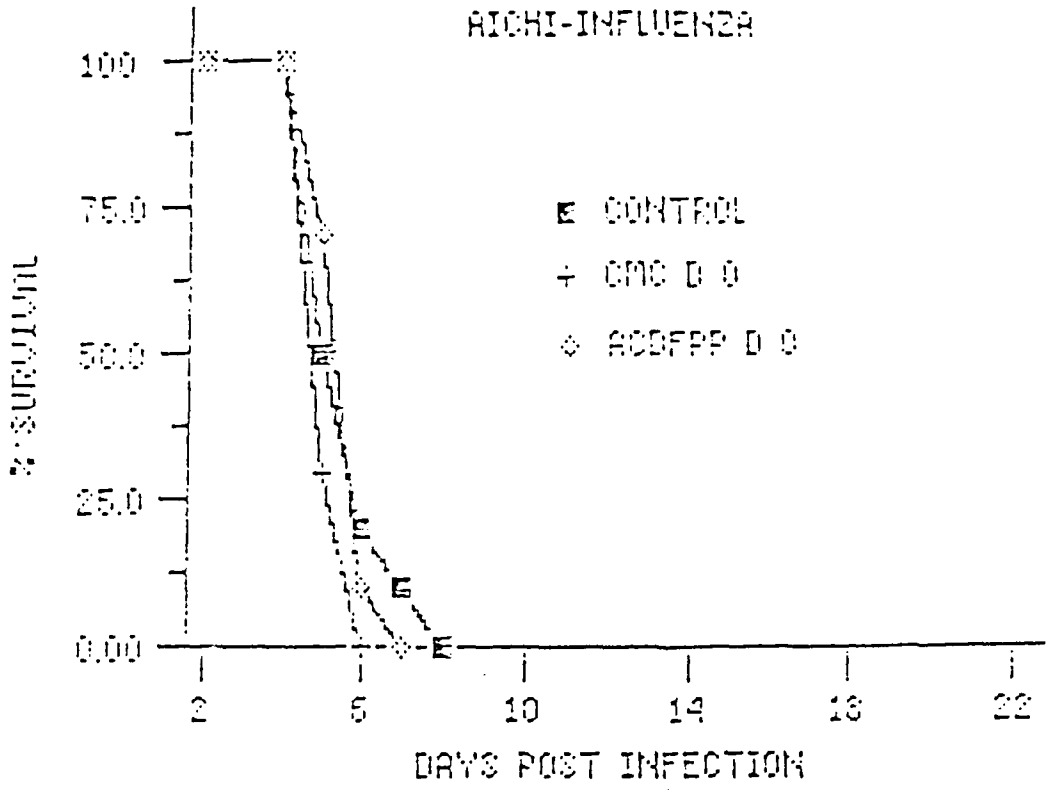
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.84	-
ACDFPP Day -2	6.03	NS
Saline Control	5.72	NS

Figure 35. Effect of ACDFPP, given on day -2, on resistance to influenza-induced pneumonitis.

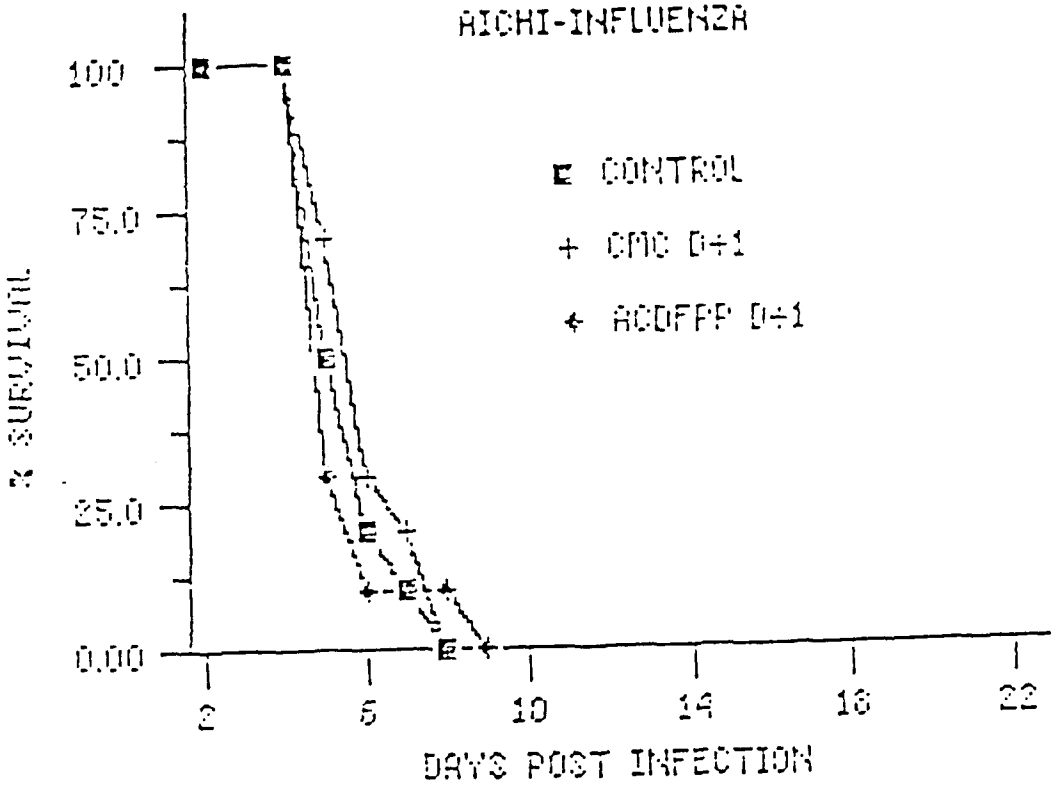
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.28	-
ACDFPP Day 0	5.77	NS
Saline Control	5.72	NS

Figure 36. Effect of ACDFPP, given on day 0, on resistance to influenza-induced pneumonitis.

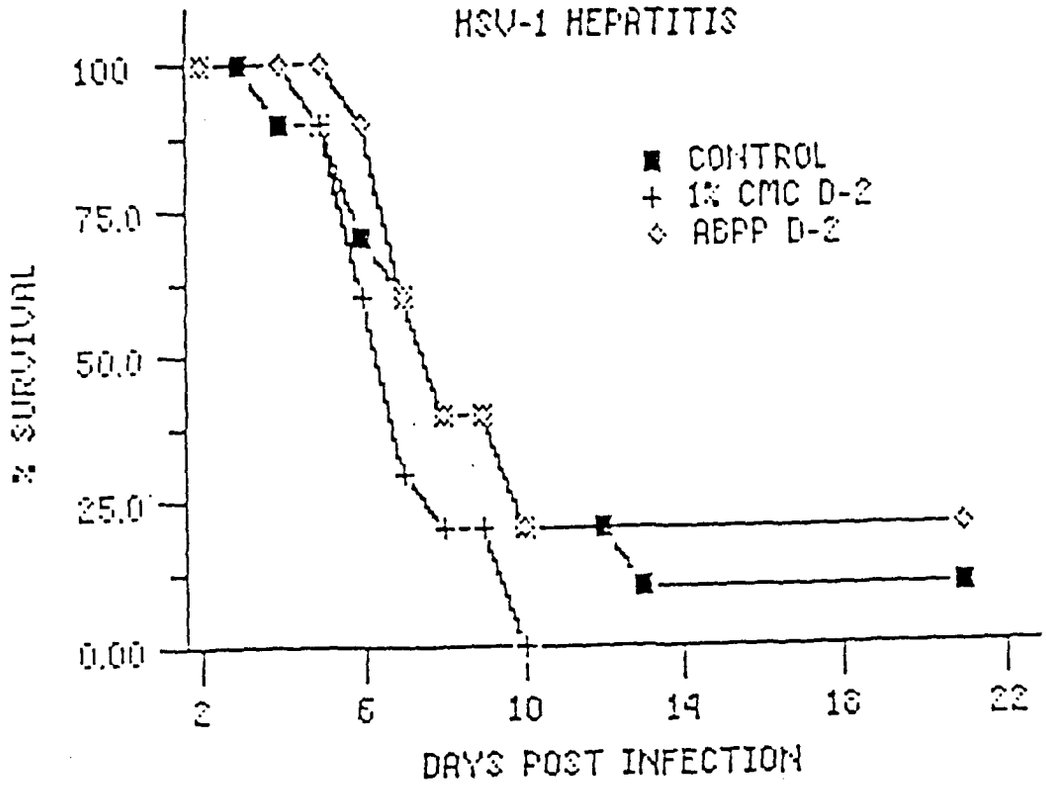
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.11	-
ACDFPP Day +1	5.50	NS
Saline Control	5.72	NS

Figure 37. Effect of ACDFPP, given on day +1, on resistance to influenza-induced pneumonitis.

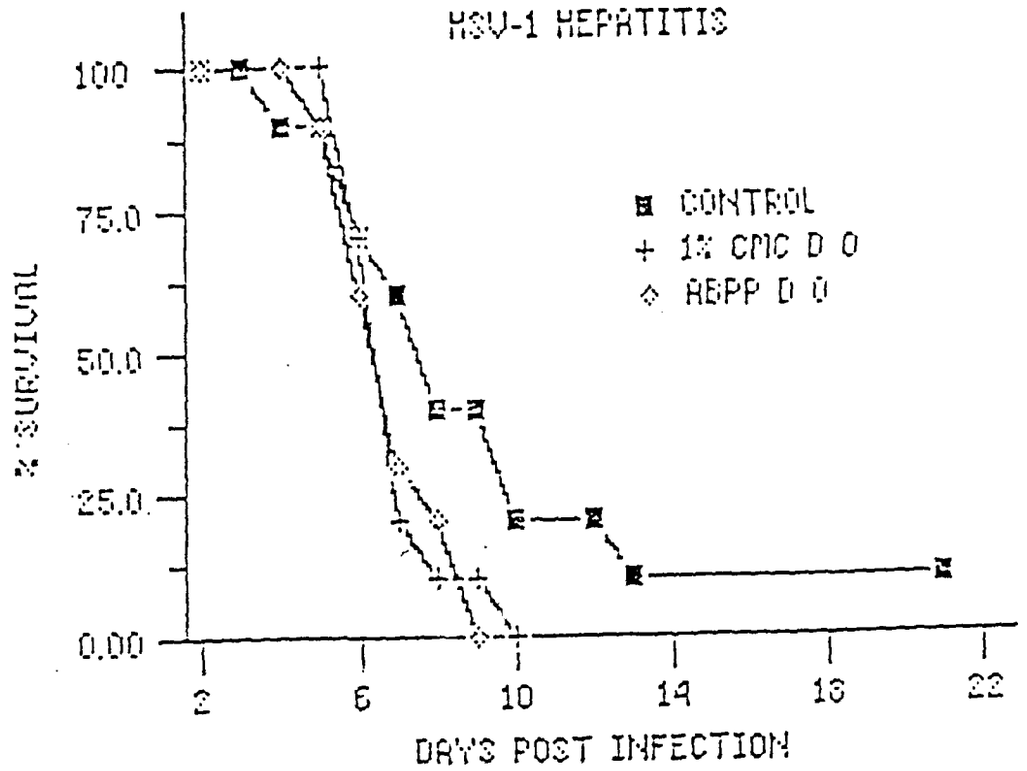
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intranasal challenge with 10 LD_{50} of influenza virus (Aichi Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.03	-
ABPP Day -2	9.47	NS
Saline Control	8.60	NS

Figure 38. Effect of ABPP, given on day -2, on resistance to herpesvirus-induced hepatitis.

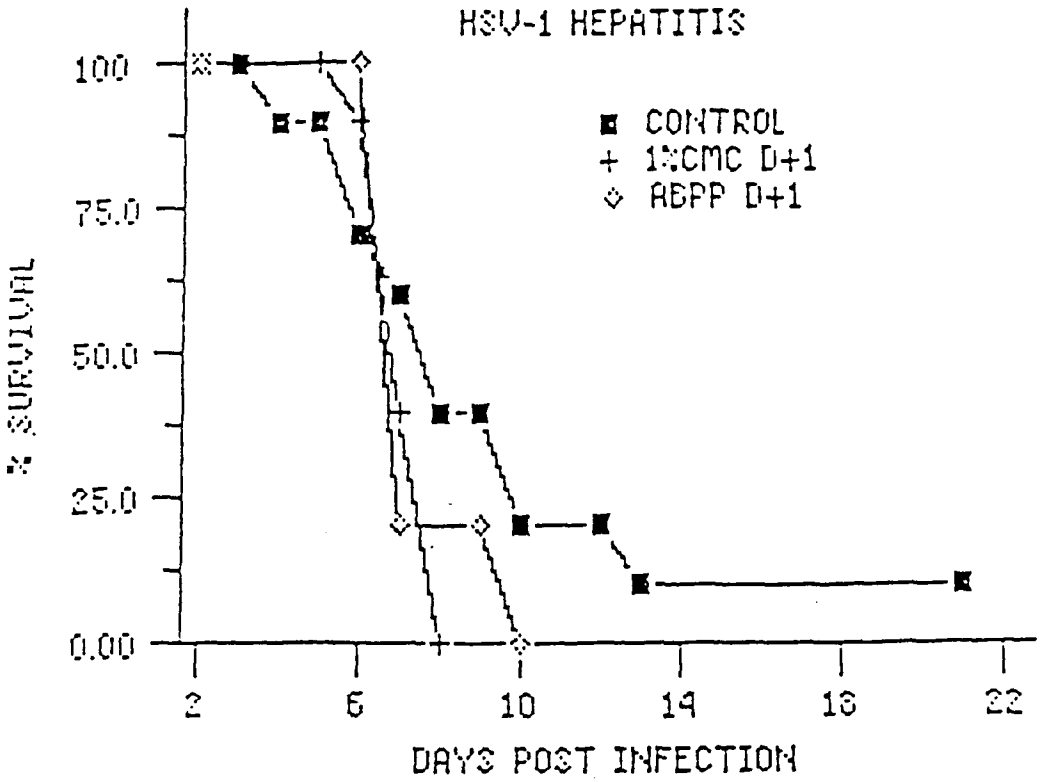
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.02	-
ABPP Day 0	6.89	<0.02
Saline Control	8.60	NS

Figure 39. *Effect of ABPP, given on day 0, on resistance to herpesvirus-induced hepatitis.*

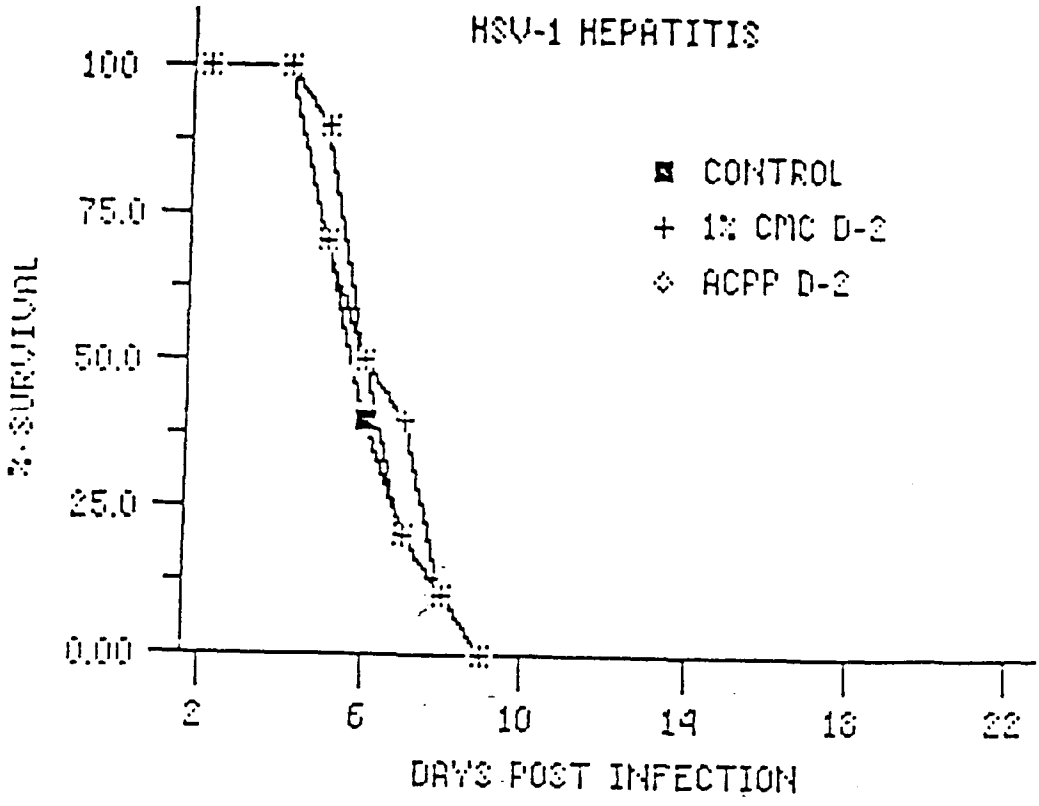
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). *Control animals received 1% CMC on D 0.* A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.27	-
ABPP Day +1	7.52	NS
Saline Control	8.60	NS

Figure 40. Effect of ABPP, given on day +1, on resistance to herpesvirus-induced hepatitis.

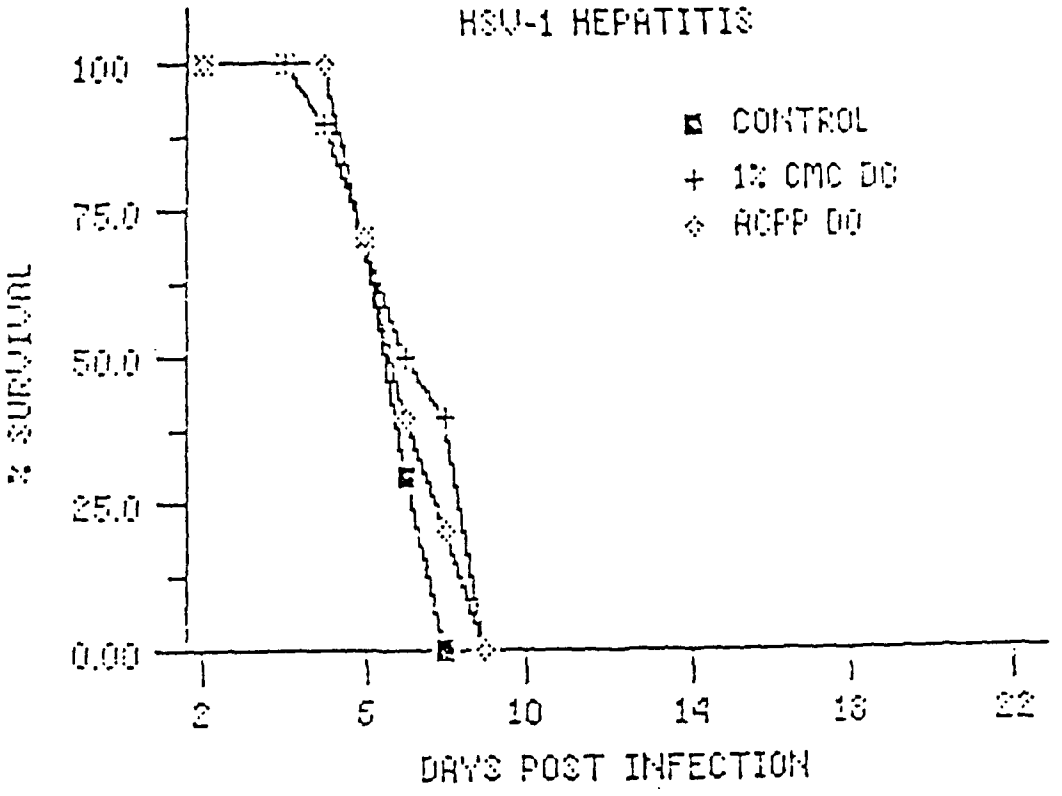
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.61	-
ACPP Day -2	6.55	NS
Saline Control	6.28	NS

Figure 41. Effect of ACPP, given on day -2, on resistance to herpesvirus-induced hepatitis.

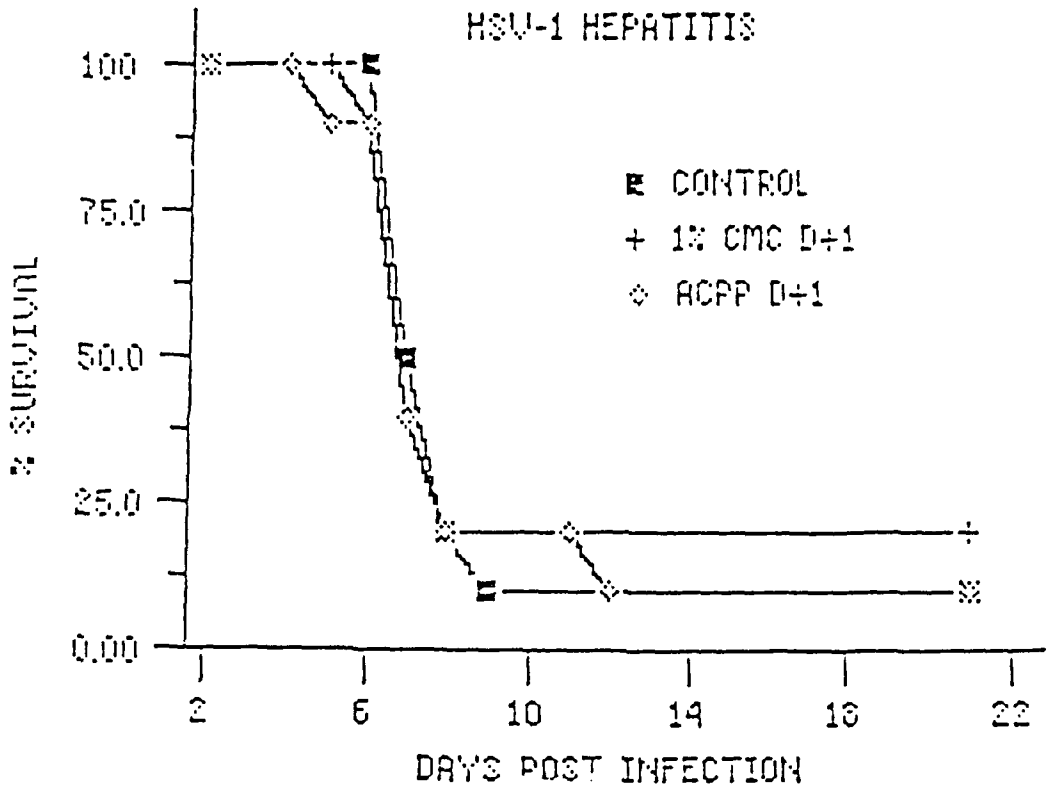
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.35	-
ACPP Day 0	7.22	NS
Saline Control	6.83	NS

Figure 42. Effect of ACPP, given on day 0, on resistance to herpesvirus-induced hepatitis.

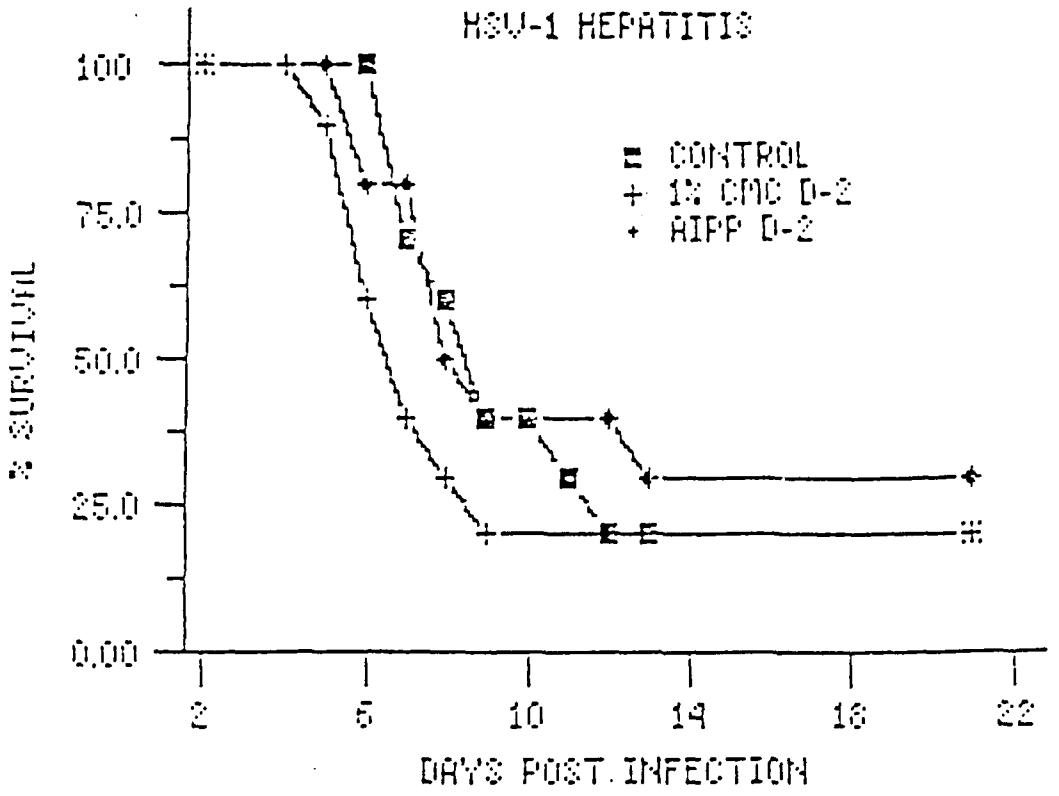
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.82	-
ACPP Day +1	8.19	NS
Saline Control	8.34	NS

Figure 43. Effect of ACPP, given on day +1, on resistance to herpesvirus-induced hepatitis.

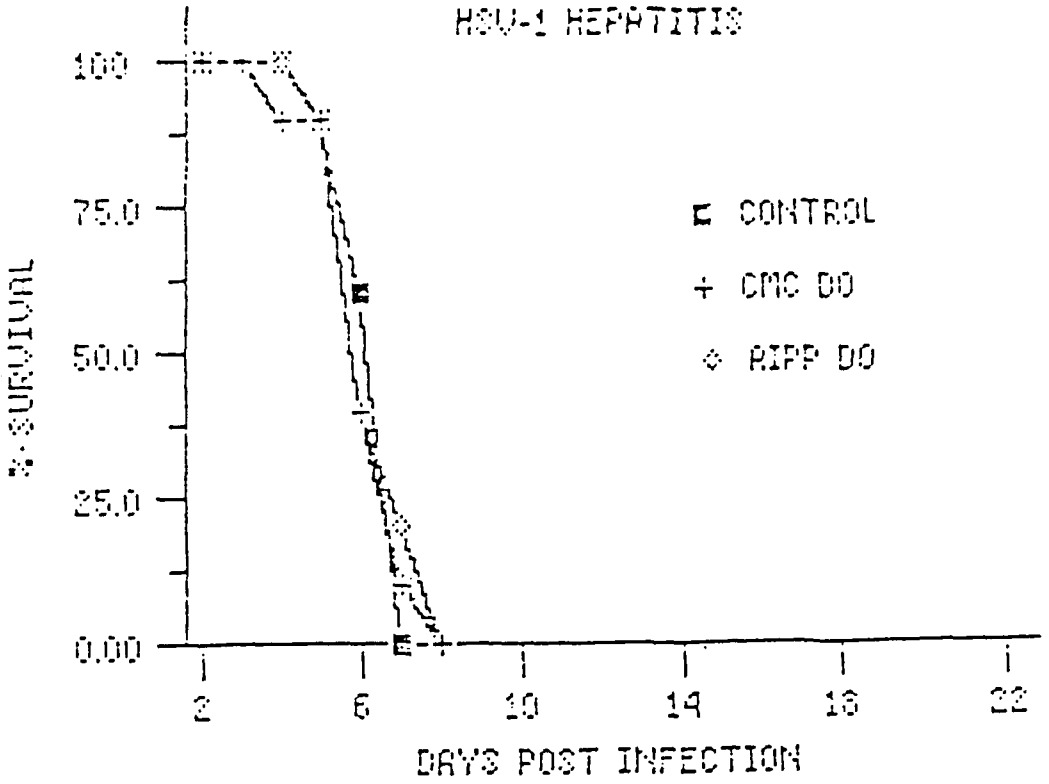
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.37	-
AIPP Day -2	10.72	NS
Saline Control	10.26	NS

Figure 44. Effect of AIPP, given on day -2, on resistance to herpesvirus-induced hepatitis.

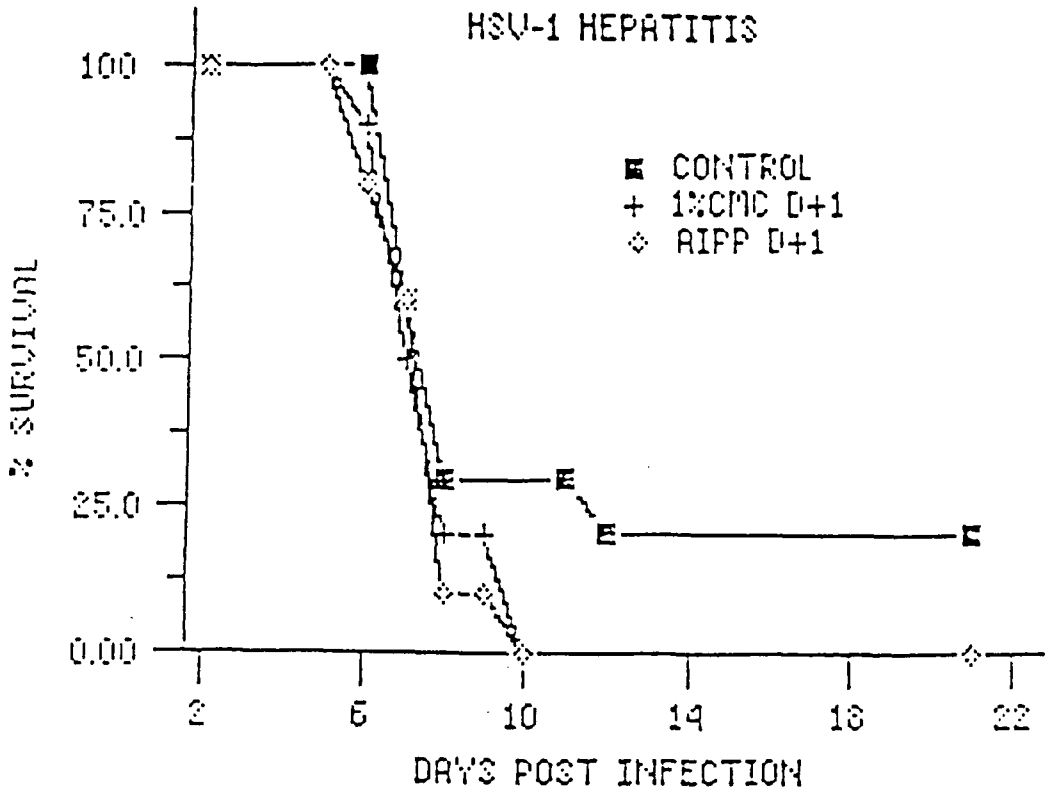
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.21	-
AIPP Day 0	6.44	NS
Saline Control	6.46	NS

Figure 45. Effect of AIPP, given on day 0, on resistance to herpesvirus-induced hepatitis.

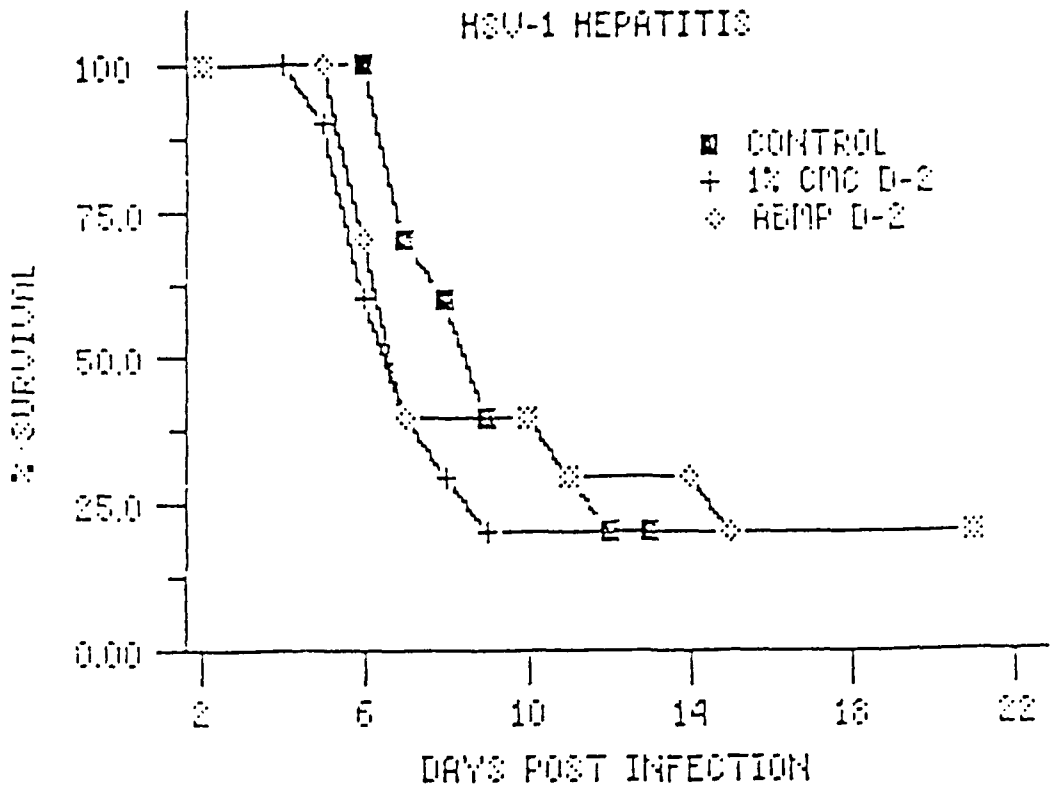
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.85	-
AIPP Day +1	7.52	NS
Saline Control	9.69	NS

Figure 46. Effect of AIPP, given on day +1, on resistance to herpesvirus-induced hepatitis.

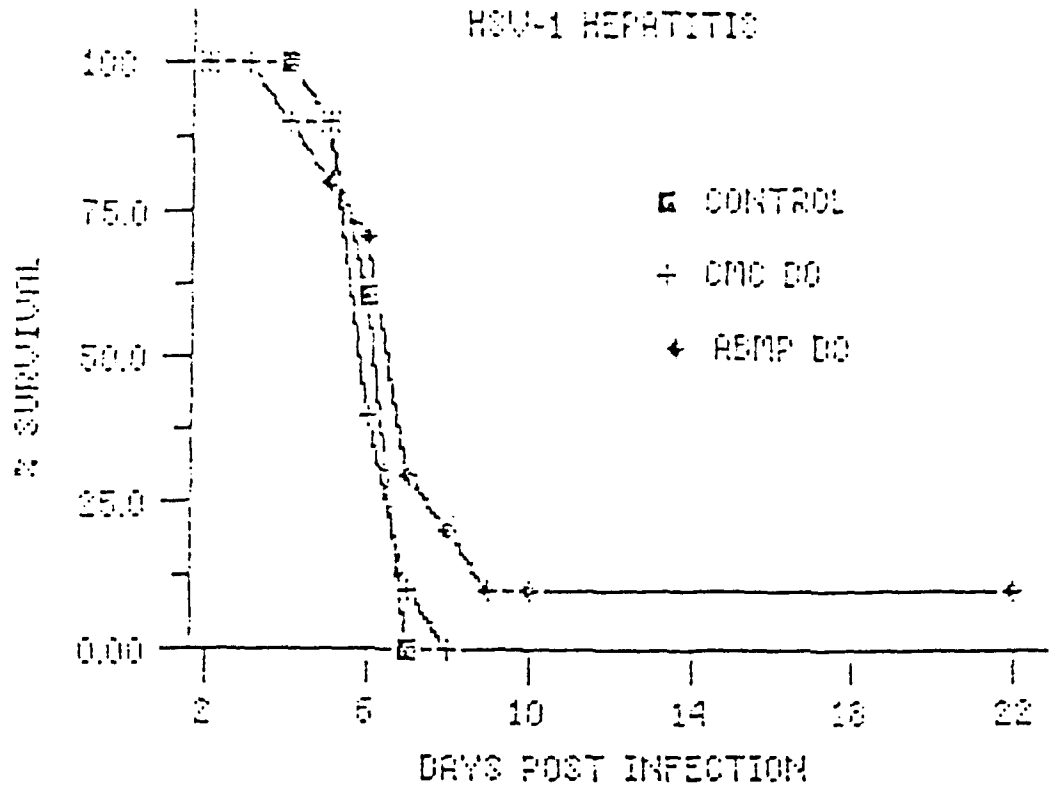
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.37	-
ABMP Day -2	9.40	NS
Saline Control	10.26	NS

Figure 47. Effect of ABMP, given on day -2, on resistance to herpesvirus-induced hepatitis.

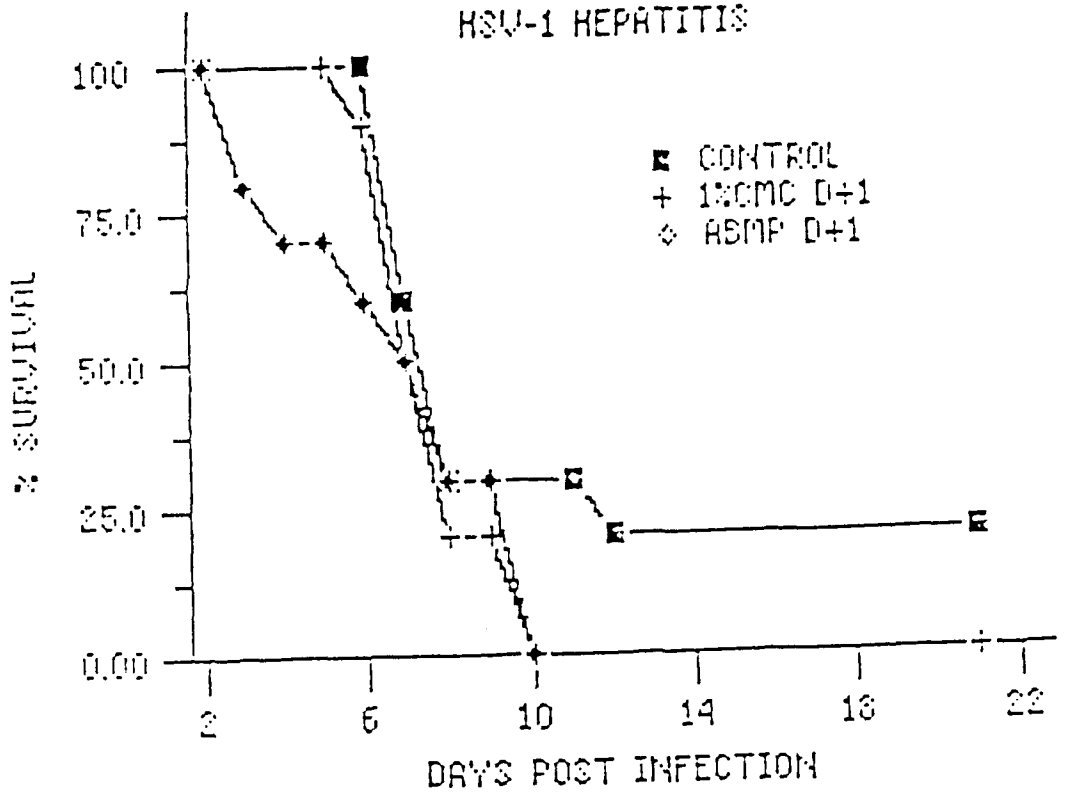
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.21	-
ABMP Day 0	7.31	NS
Saline Control	6.46	NS

Figure 48. Effect of ABMP, given on day 0, on resistance to herpesvirus-induced hepatitis.

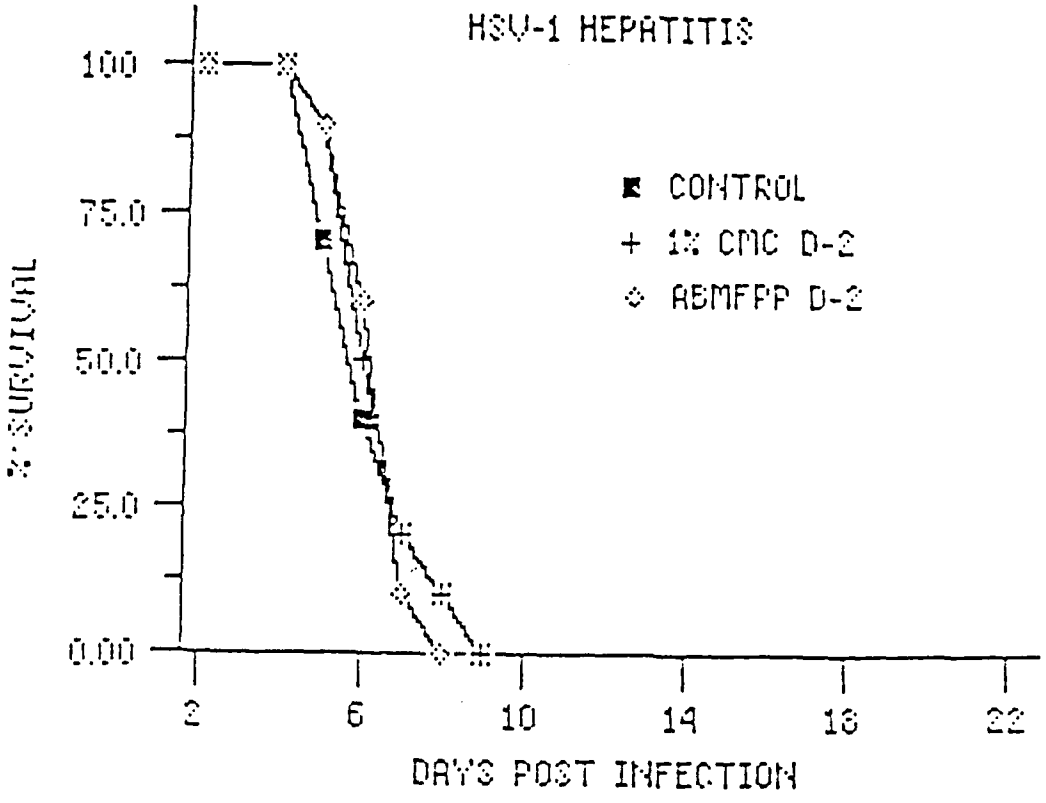
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.85	-
ABMP Day +1	5.63	NS
Saline Control	9.69	NS

Figure 49. Effect of ABMP, given on day +1, on resistance to herpesvirus-induced hepatitis.

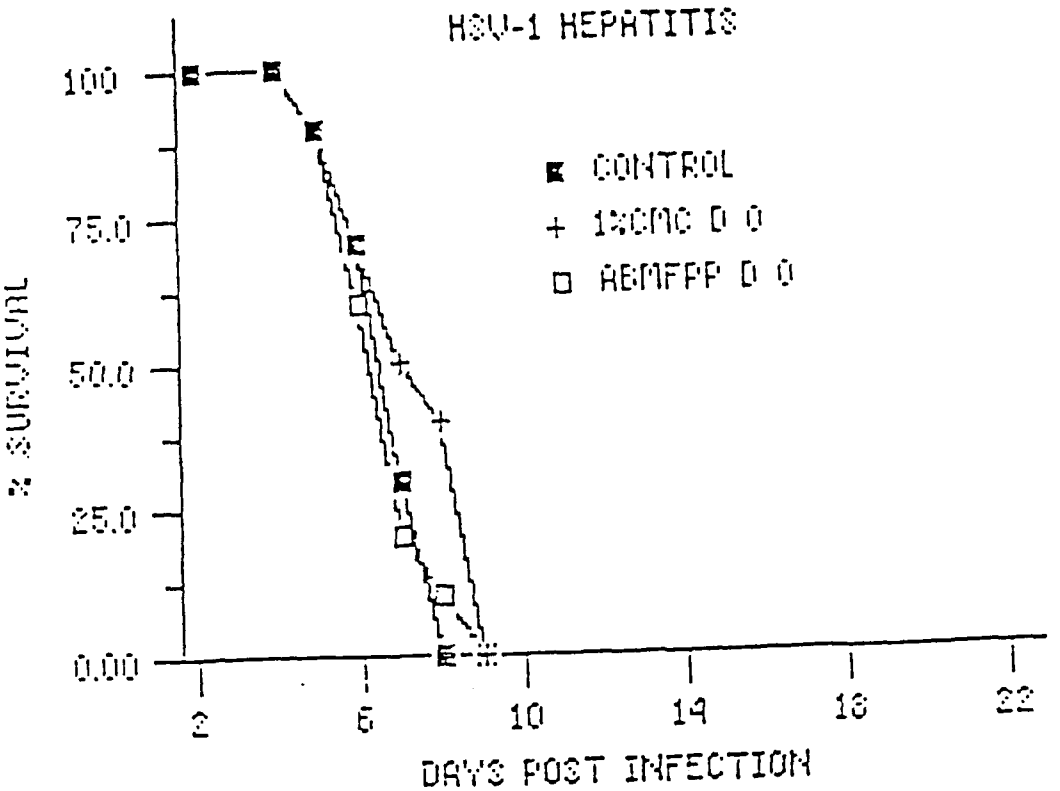
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.61	-
ABMFPP Day -2	6.65	NS
Saline Control	6.28	NS

Figure 50. Effect of ABMFPP, given on day -2, on resistance to herpesvirus-induced hepatitis.

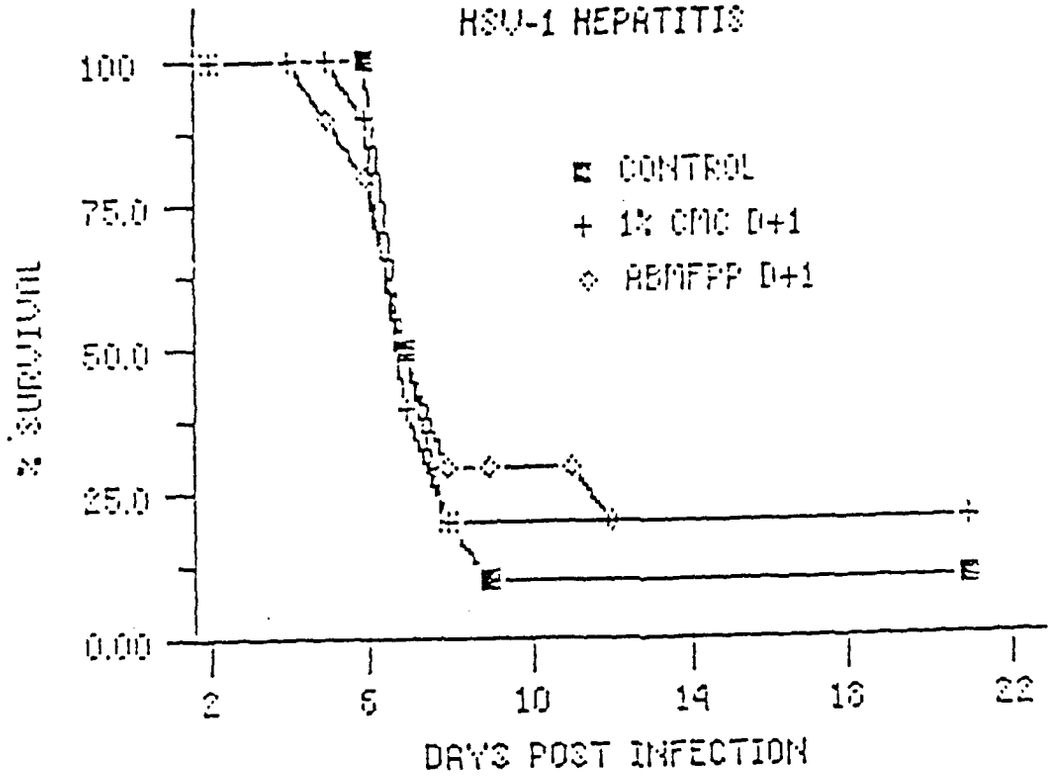
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.35	-
ABMFPP Day 0	6.72	NS
Saline Control	6.83	NS

Figure 51. Effect of ABMFPP, given on day 0, on resistance to herpesvirus-induced hepatitis.

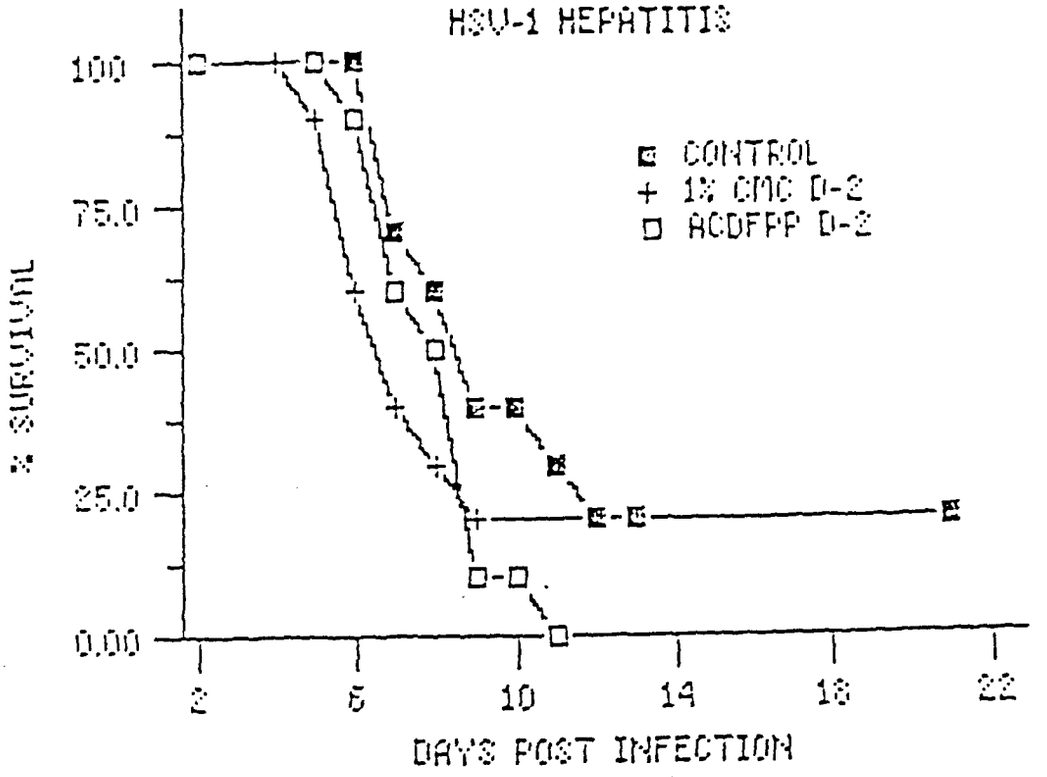
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.82	-
ABMFPP Day +1	9.17	NS
Saline Control	8.34	NS

Figure 52. Effect of ABMFPP, given on day +1, on resistance to herpesvirus-induced hepatitis.

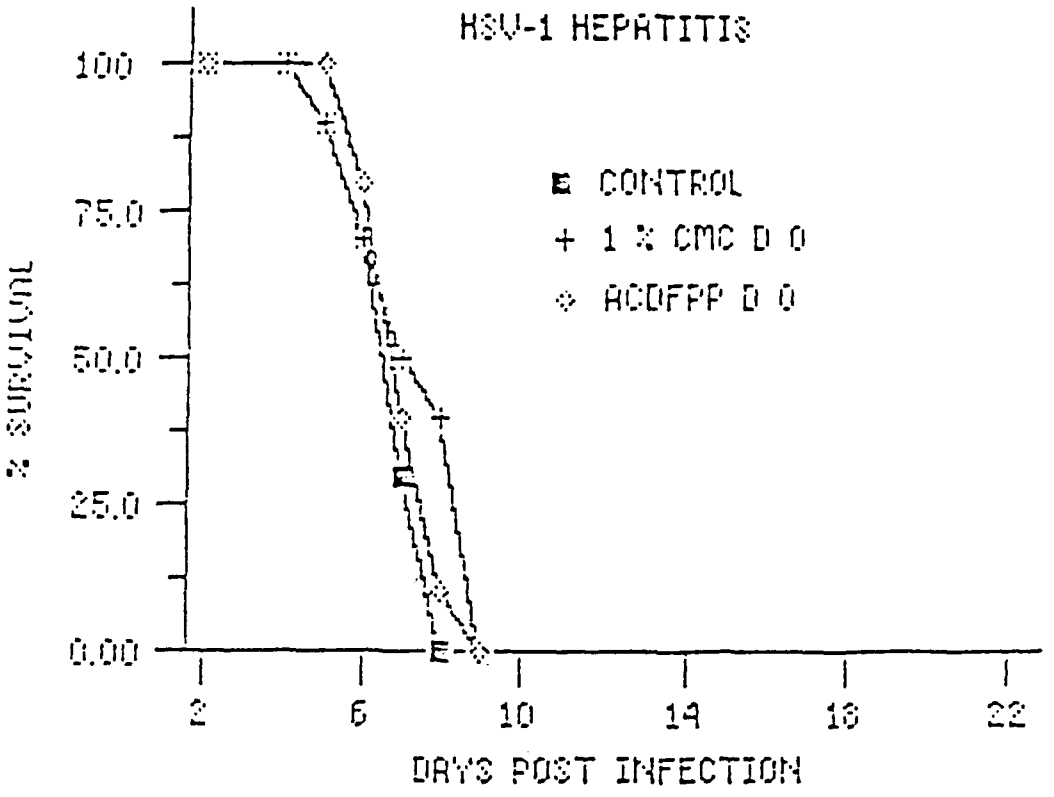
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.37	-
ACDFPP Day -2	8.08	NS
Saline Control	10.26	NS

Figure 53. Effect of ACDFPP, given on day -2, on resistance to herpesvirus-induced hepatitis.

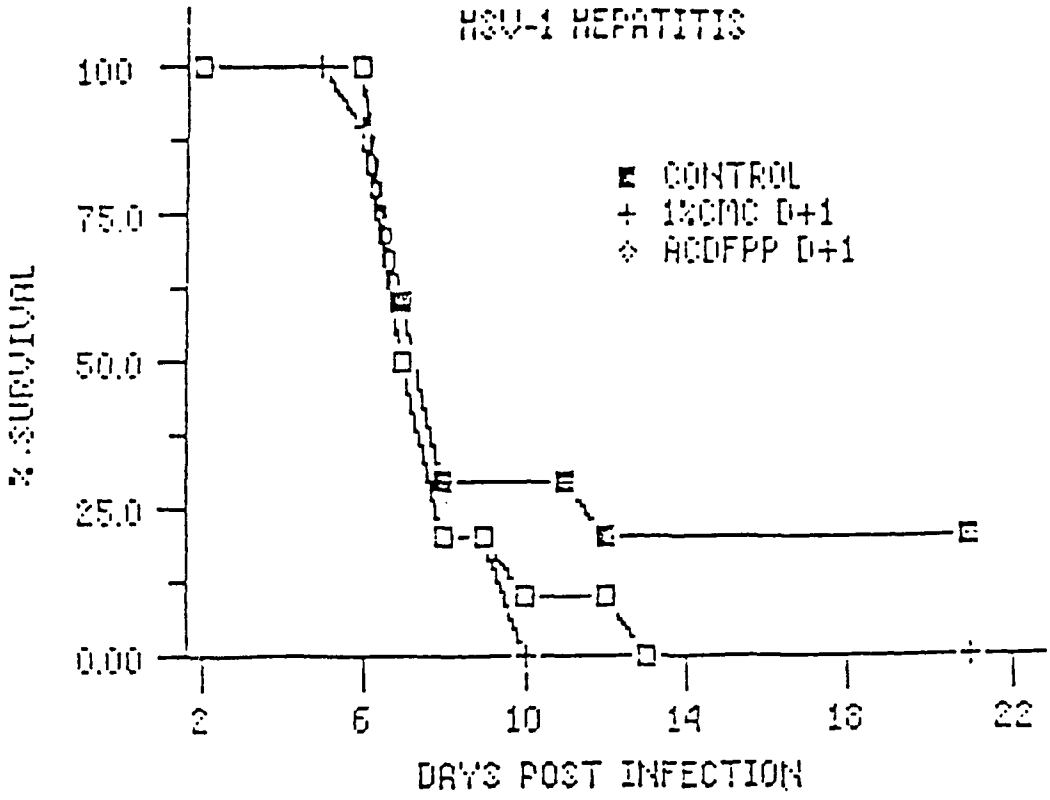
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.35	-
ACDFPP Day 0	7.24	NS
Saline Control	6.83	NS

Figure 54. Effect of ACDFPP, given on day 0, on resistance to herpesvirus-induced hepatitis.

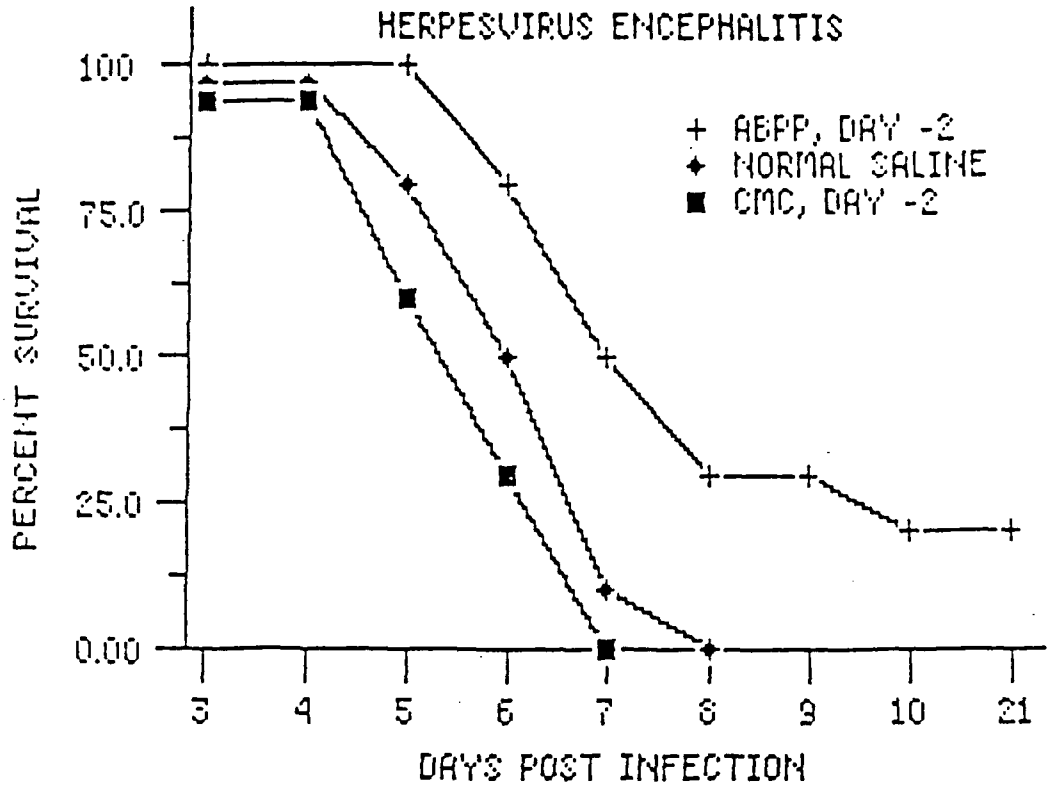
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.85	-
ACDFPP Day +1	8.03	NS
Saline Control	9.69	NS

Figure 55. Effect of ACDFPP, given on day +1, on resistance to herpesvirus-induced hepatitis.

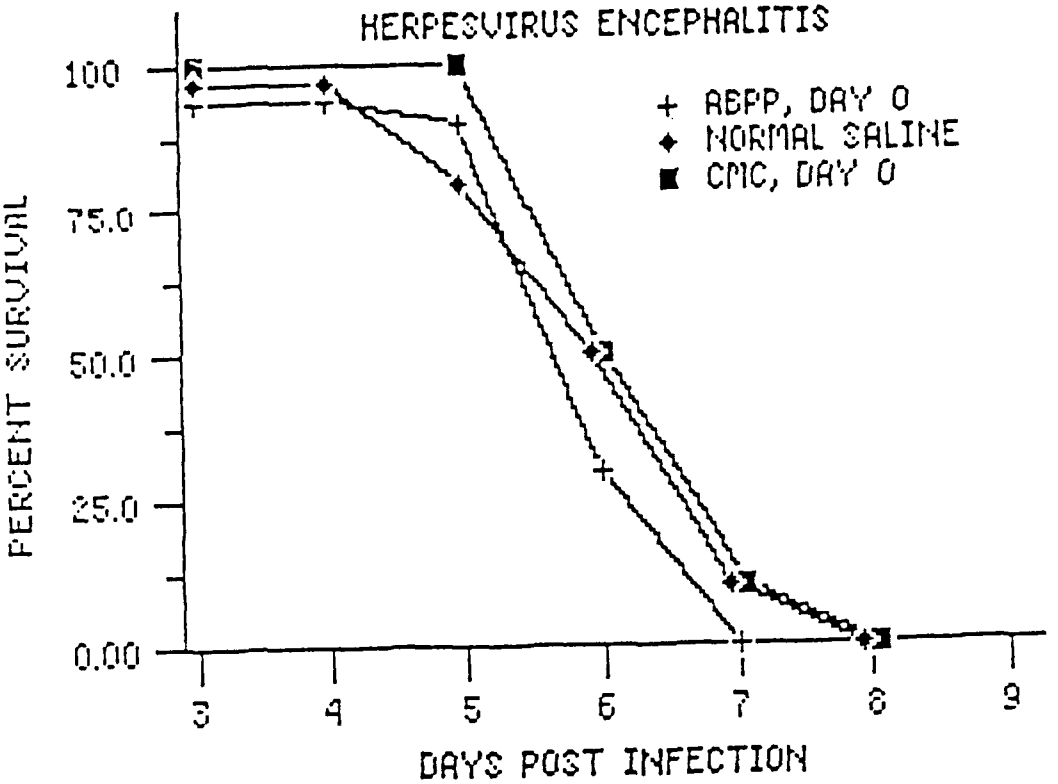
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) intravenous challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.84	-
ABPP Day -2	7.82	<0.01
Saline Control	6.33	NS

Figure 56. Effect of ABPP, given on day -2, on resistance to herpesvirus-induced encephalitis.

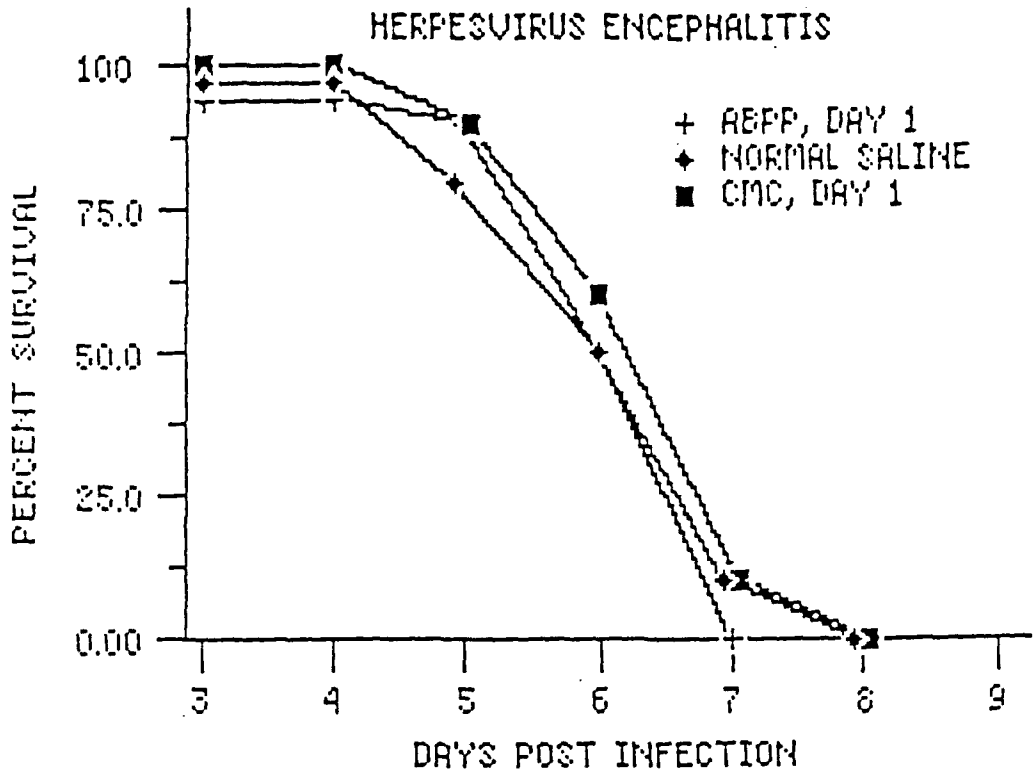
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.57	-
ABPP Day 0	6.17	NS
Saline Control	6.33	NS

Figure 57. Effect of ABPP, given on day 0, on resistance to herpesvirus-induced encephalitis.

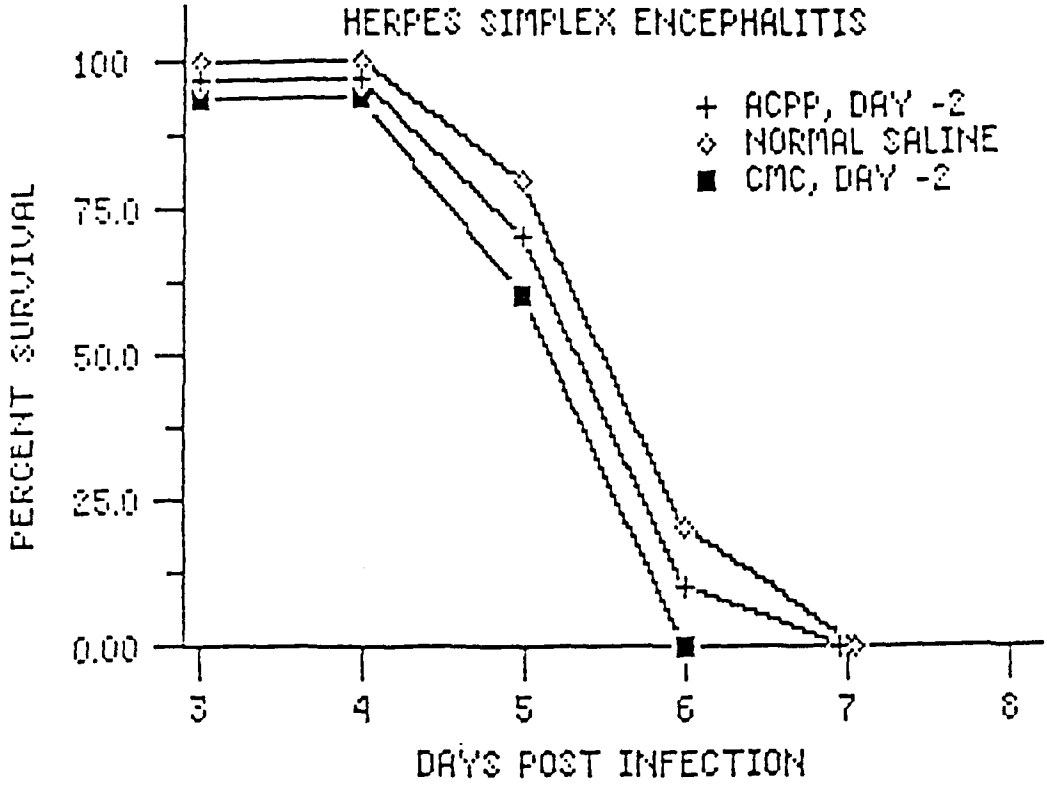
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.55	-
ABPP Day +1	6.36	NS
Saline Control	6.33	NS

Figure 58. Effect of ABPP, given on day +1, on resistance to herpesvirus-induced encephalitis.

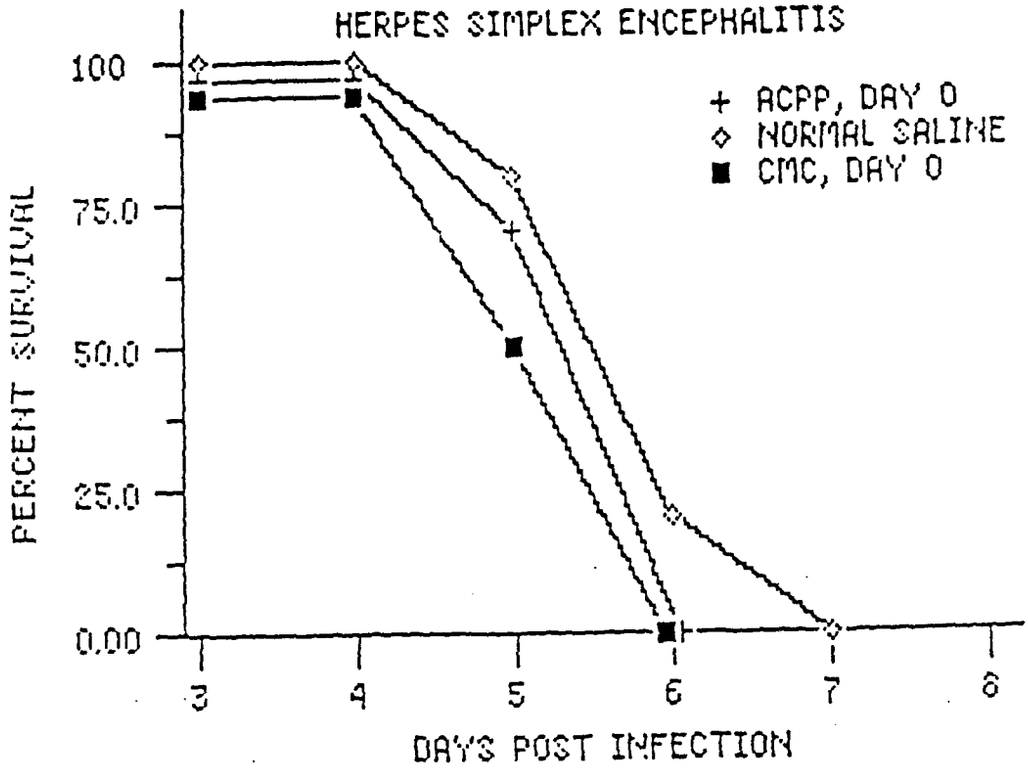
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.58	-
ACPP Day -2	5.77	NS
Saline Control	5.97	NS

Figure 59. Effect of ACPP, given on day -2, on resistance to herpesvirus-induced encephalitis.

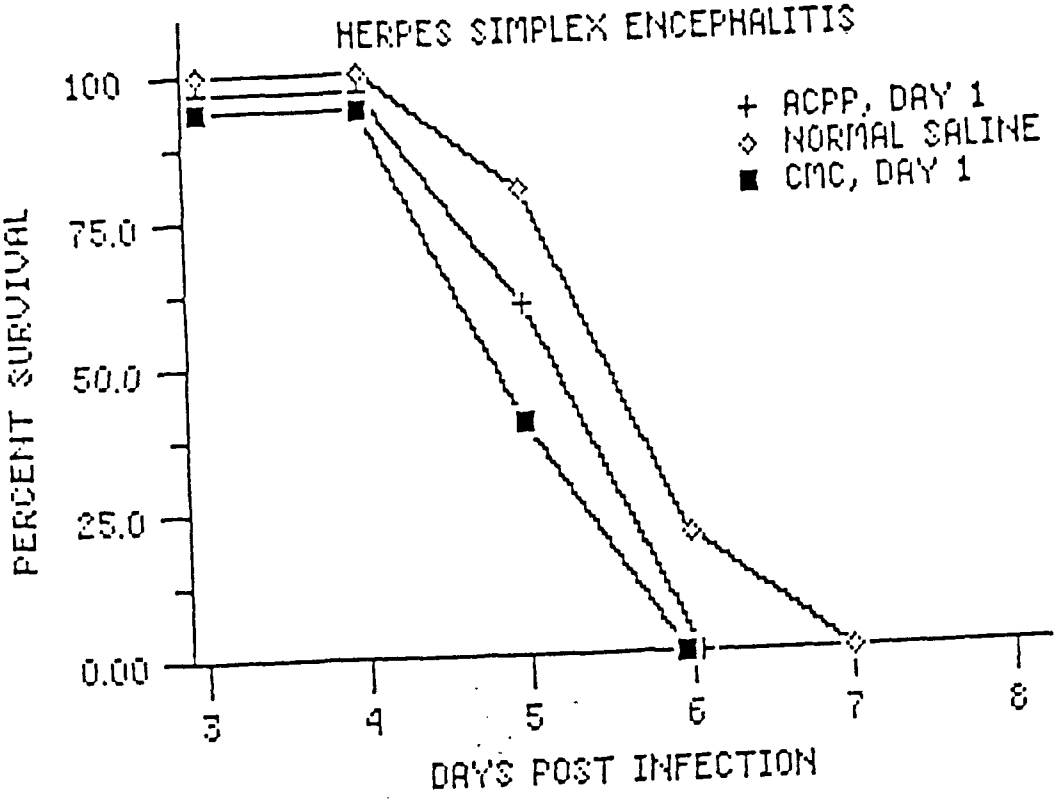
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.48	-
ACPP Day 0	5.68	NS
Saline Control	5.97	NS

Figure 60. Effect of ACPP, given on day 0, on resistance to herpesvirus-induced encephalitis.

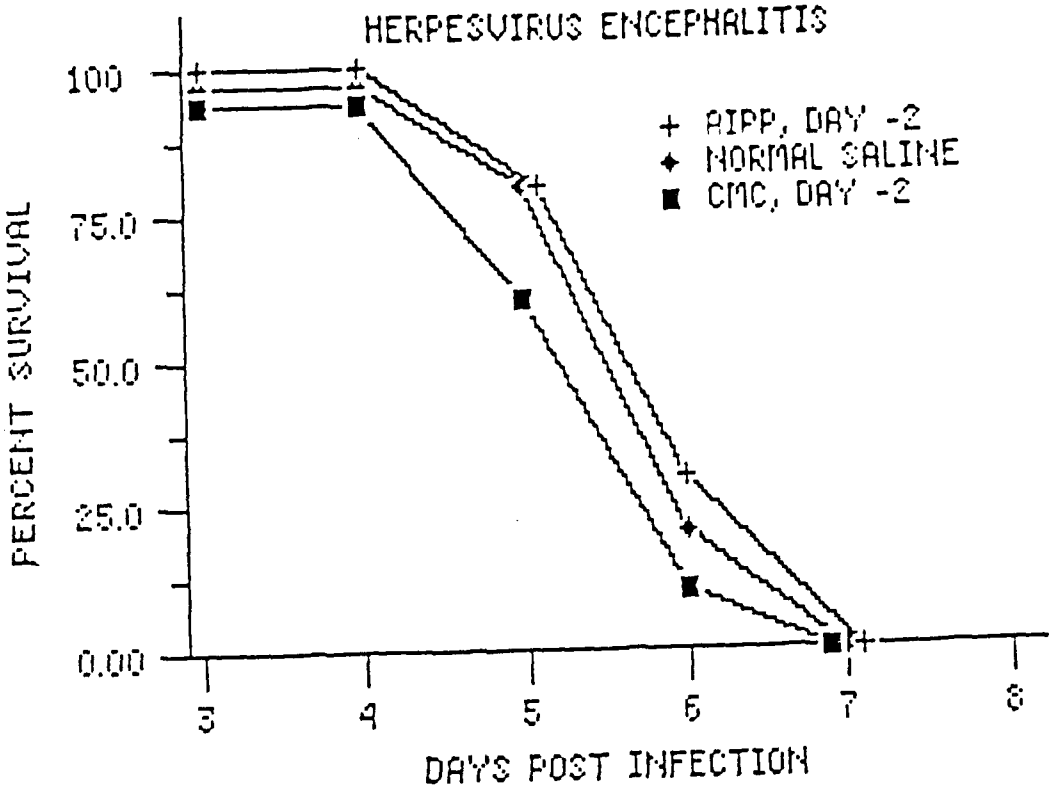
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.38	-
ACPP Day +1	5.58	NS
Saline Control	5.97	NS

Figure 61. Effect of ACPP, given on day +1, on resistance to herpesvirus-induced encephalitis.

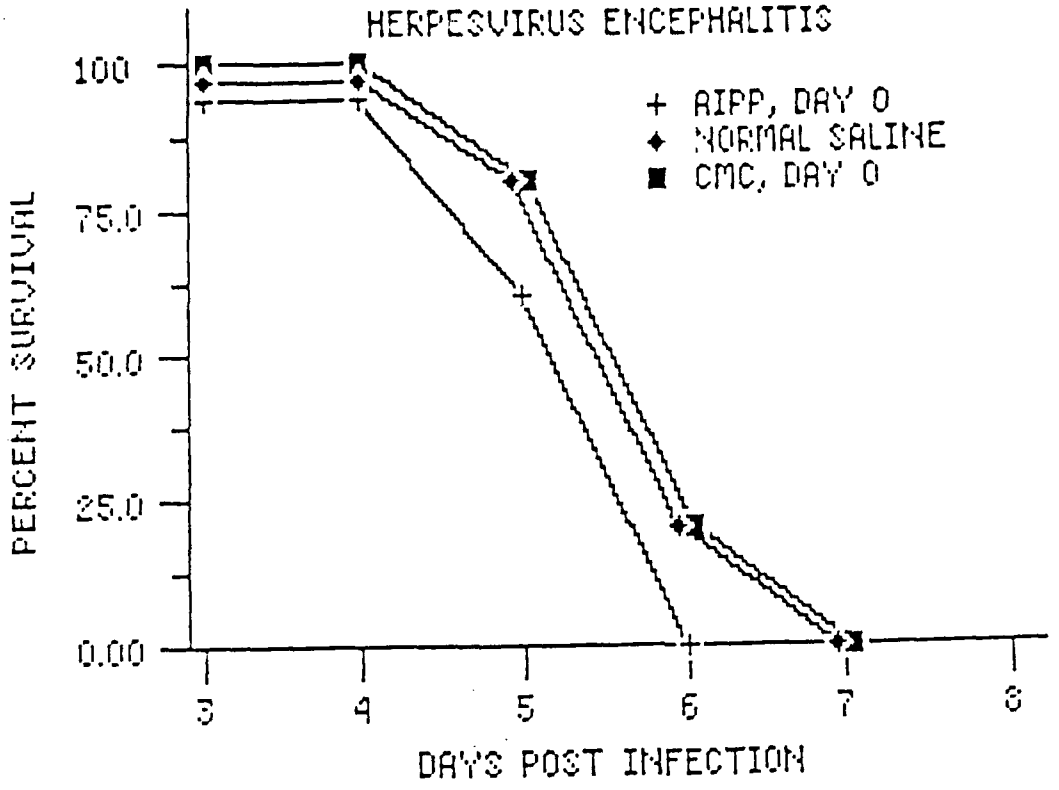
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.66	-
AIPP Day -2	6.06	NS
Saline Control	5.97	NS

Figure 62. Effect of AIPP, given on day -2, on resistance to herpesvirus-induced encephalitis.

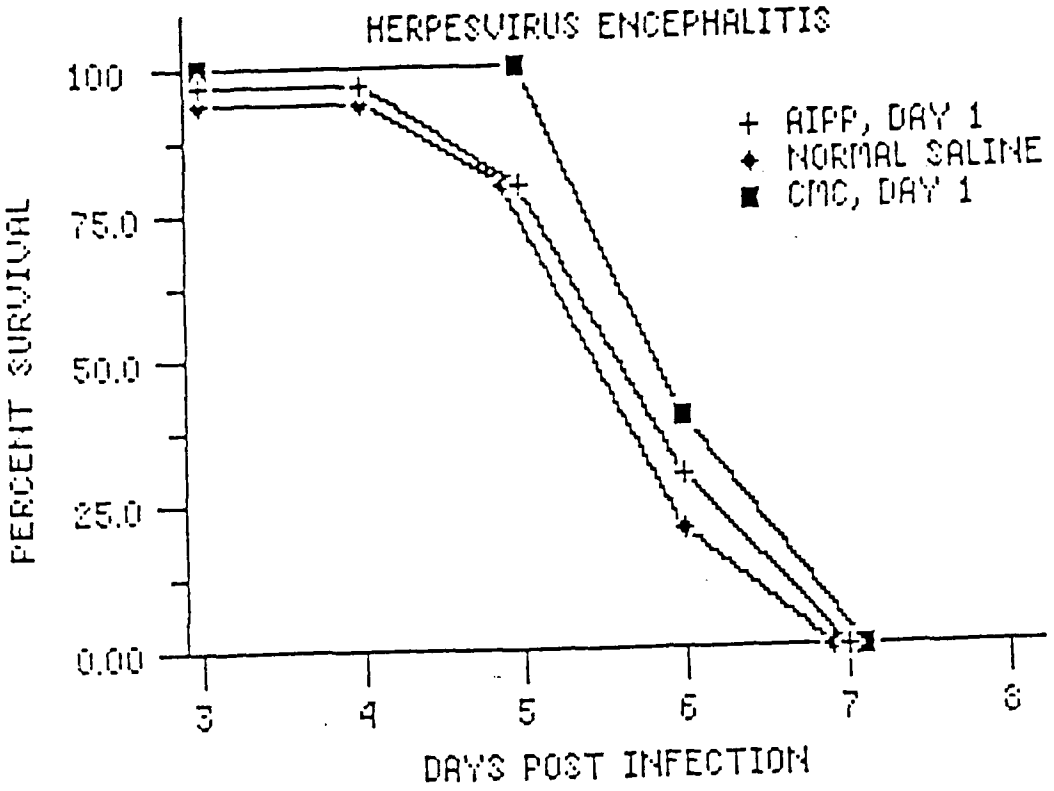
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.97	-
AIPP Day 0	5.58	NS
Saline Control	5.97	NS

Figure 63. Effect of AIPP, given on day 0, on resistance to herpesvirus-induced encephalitis.

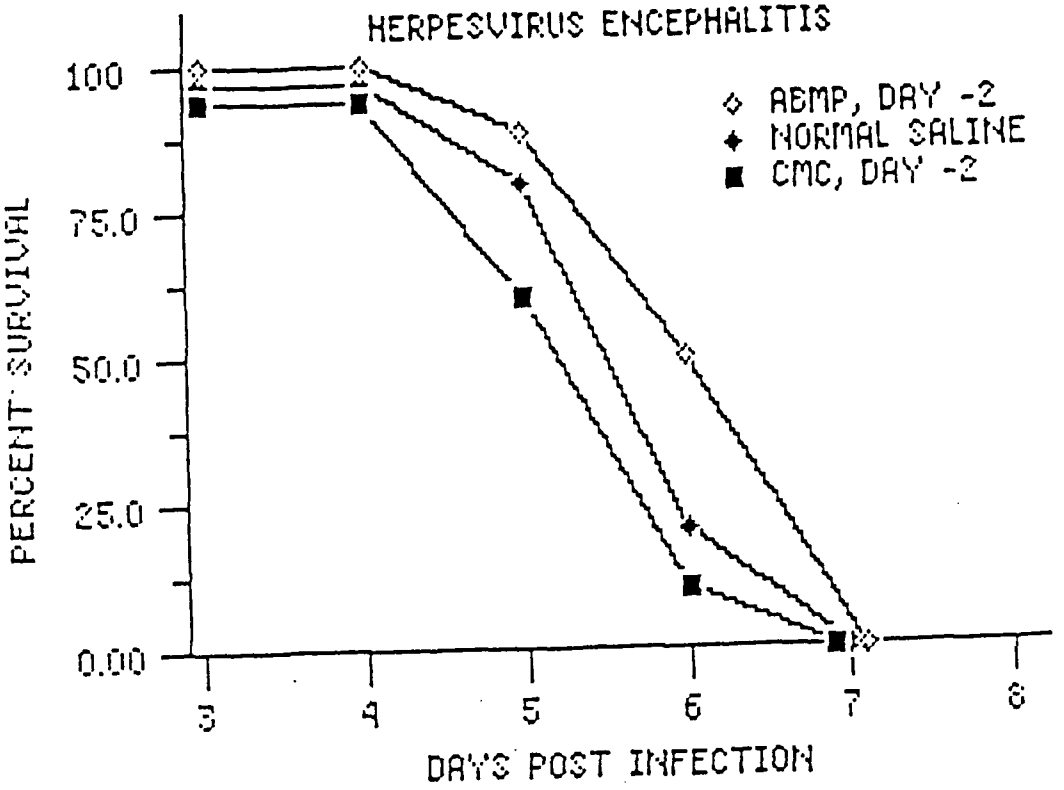
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.38	-
AIPP Day +1	6.06	NS
Saline Control	5.97	NS

Figure 64. Effect of AIPP, given on day +1, on resistance to herpesvirus-induced encephalitis.

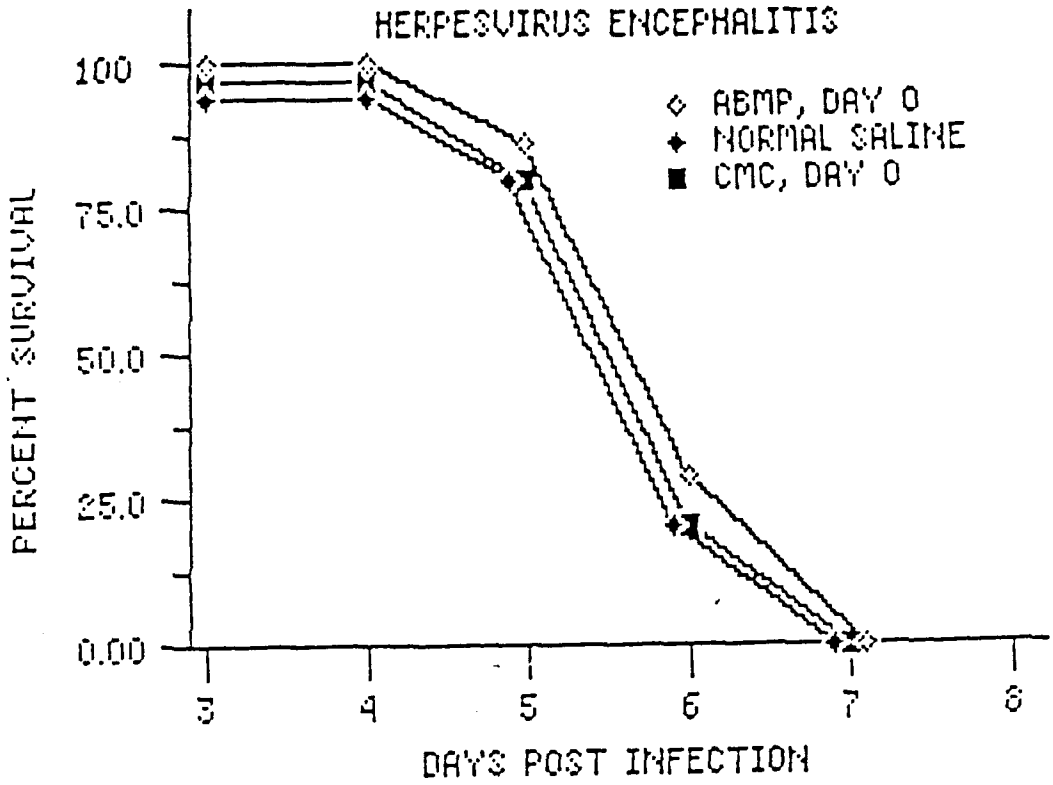
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.66	-
ABMP Day -2	6.30	NS
Saline Control	5.97	NS

Figure 65. Effect of ABMP, given on day -2, on resistance to herpesvirus-induced encephalitis.

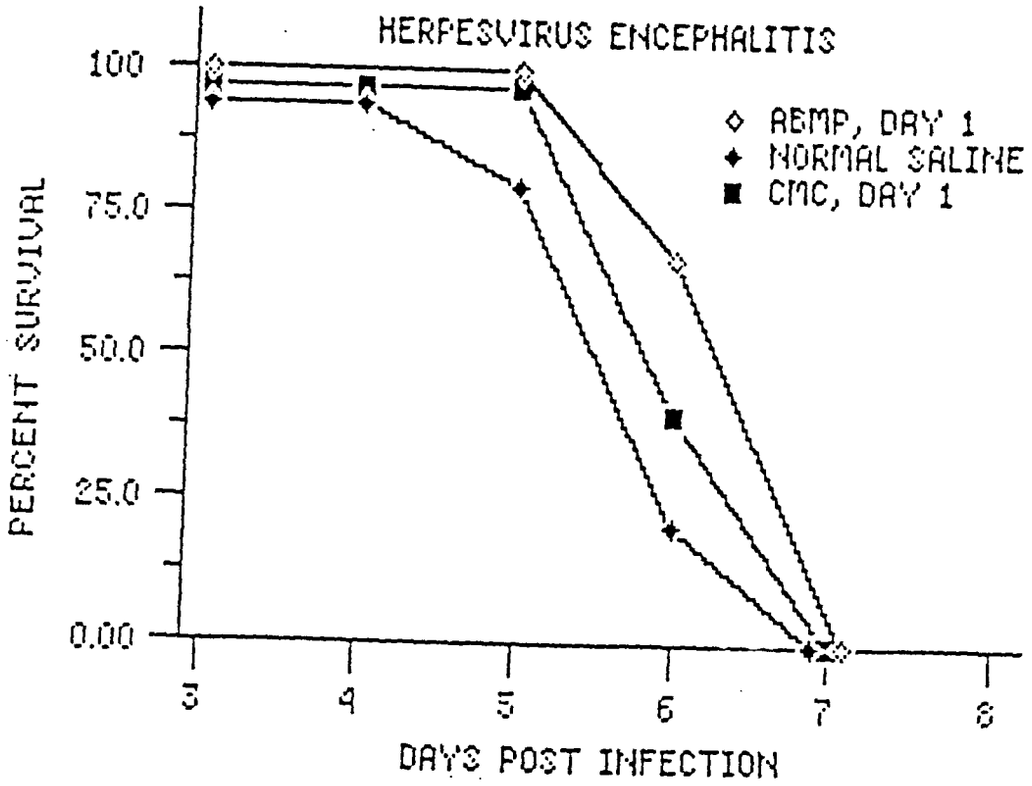
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.97	-
ABMP Day 0	6.11	NS
Saline Control	5.97	NS

Figure 66. Effect of ABMP, given on day 0, on resistance to herpesvirus-induced encephalitis.

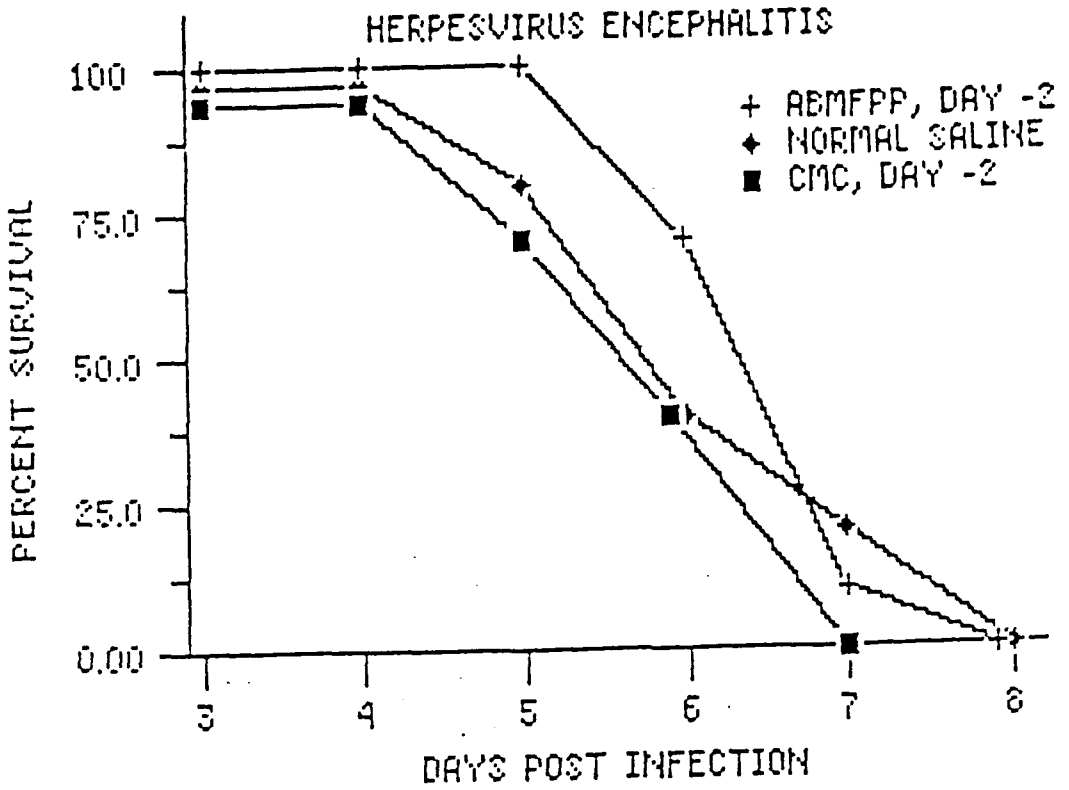
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.38	-
ABMP Day +1	6.32	NS
Saline Control	5.97	NS

Figure 67. Effect of ABMP, given on day +1, on resistance to herpesvirus-induced encephalitis.

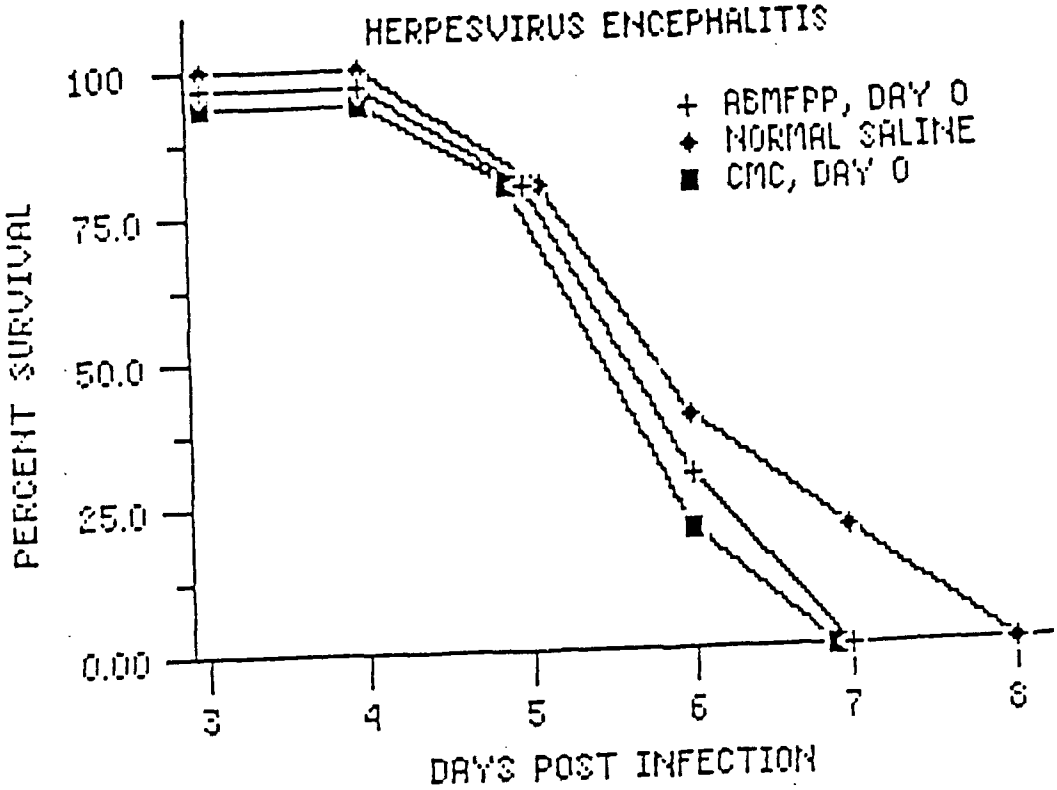
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.04	-
ABMFPP Day -2	6.77	NS
Saline Control	6.32	NS

Figure 68. Effect of ABMFPP, given on day -2, on resistance to herpesvirus-induced encephalitis.

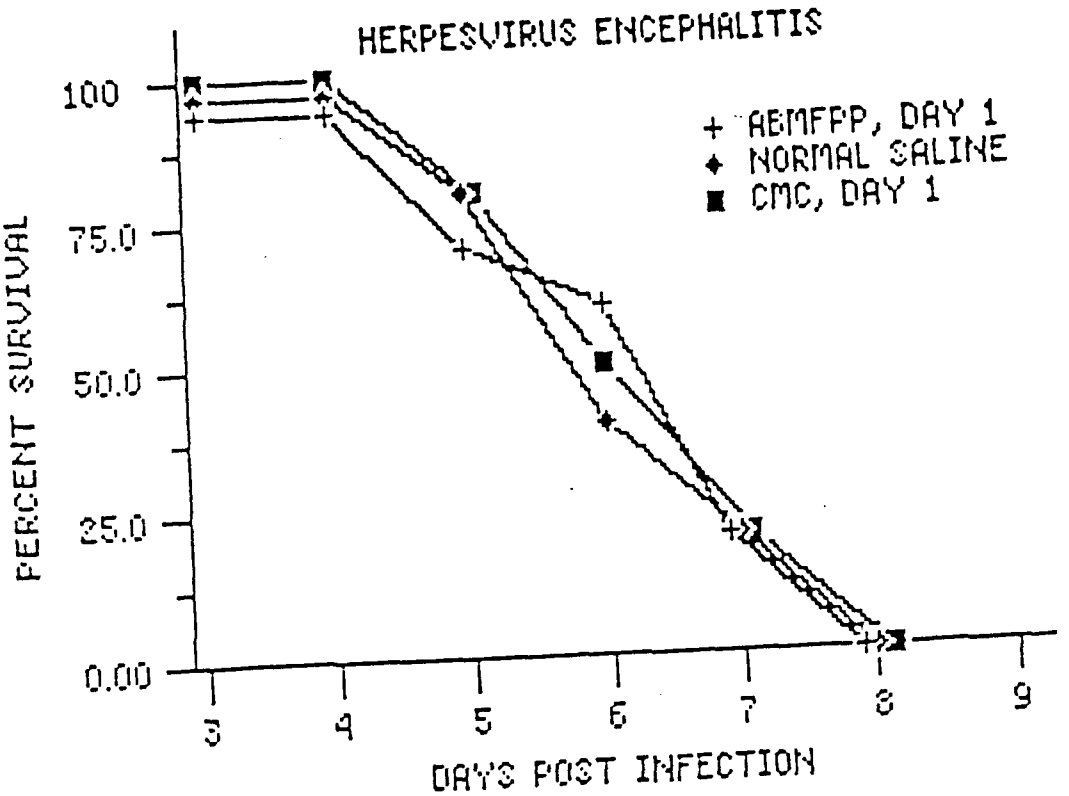
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.97	-
ABMFPP Day 0	6.06	NS
Saline Control	6.32	NS

Figure 69. Effect of ABMFPP, given on day 0, on resistance to herpesvirus-induced encephalitis.

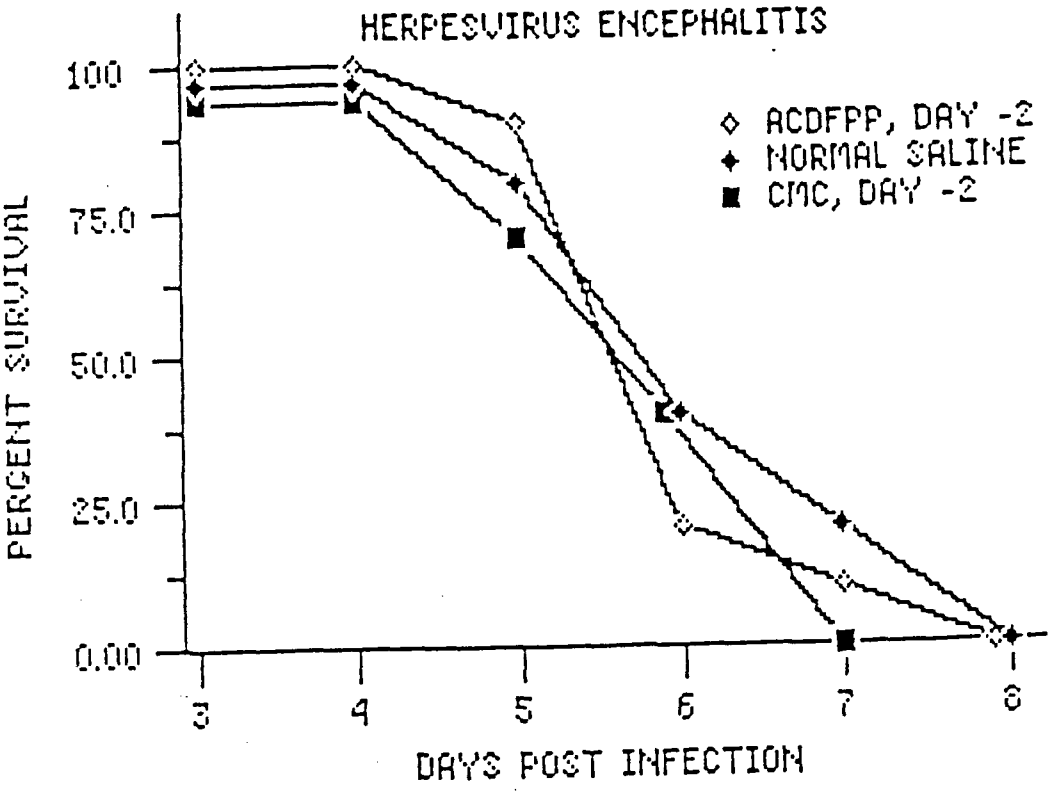
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.42	-
ABMFPP Day +1	6.40	NS
Saline Control	6.32	NS

Figure 70. Effect of ABMFPP, given on day +1, on resistance to herpesvirus-induced encephalitis.

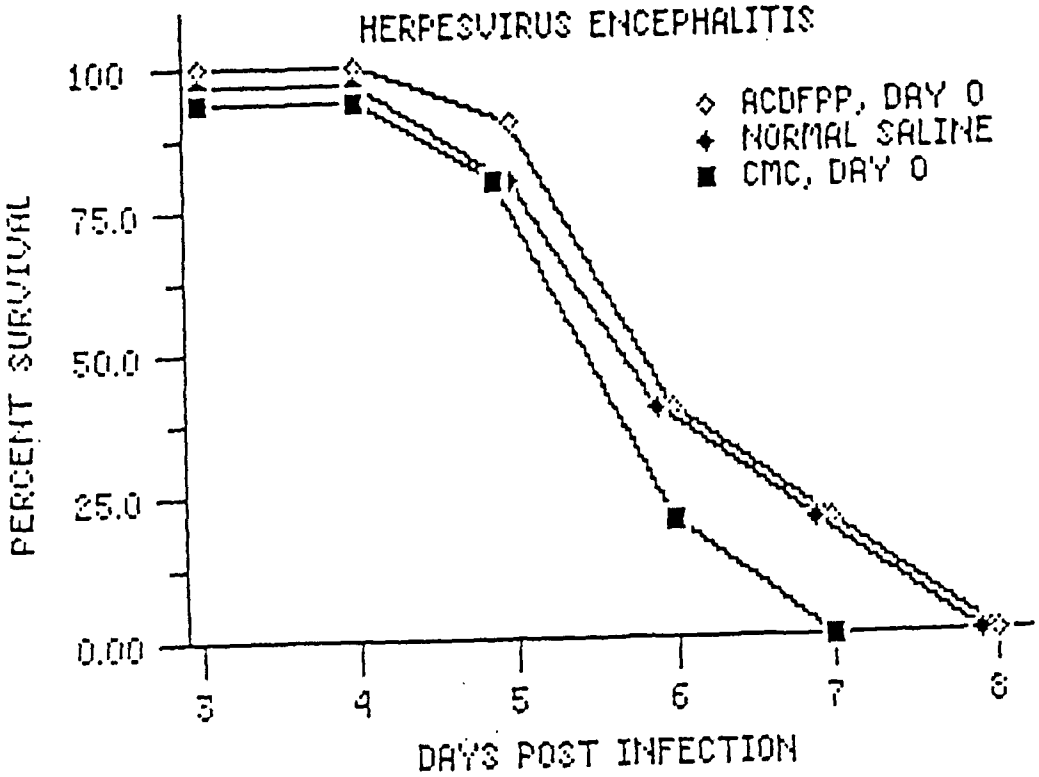
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.04	-
ACDFPP Day -2	6.16	NS
Saline Control	6.32	NS

Figure 71. Effect of ACDFPP, given on day -2, on resistance to herpesvirus-induced encephalitis.

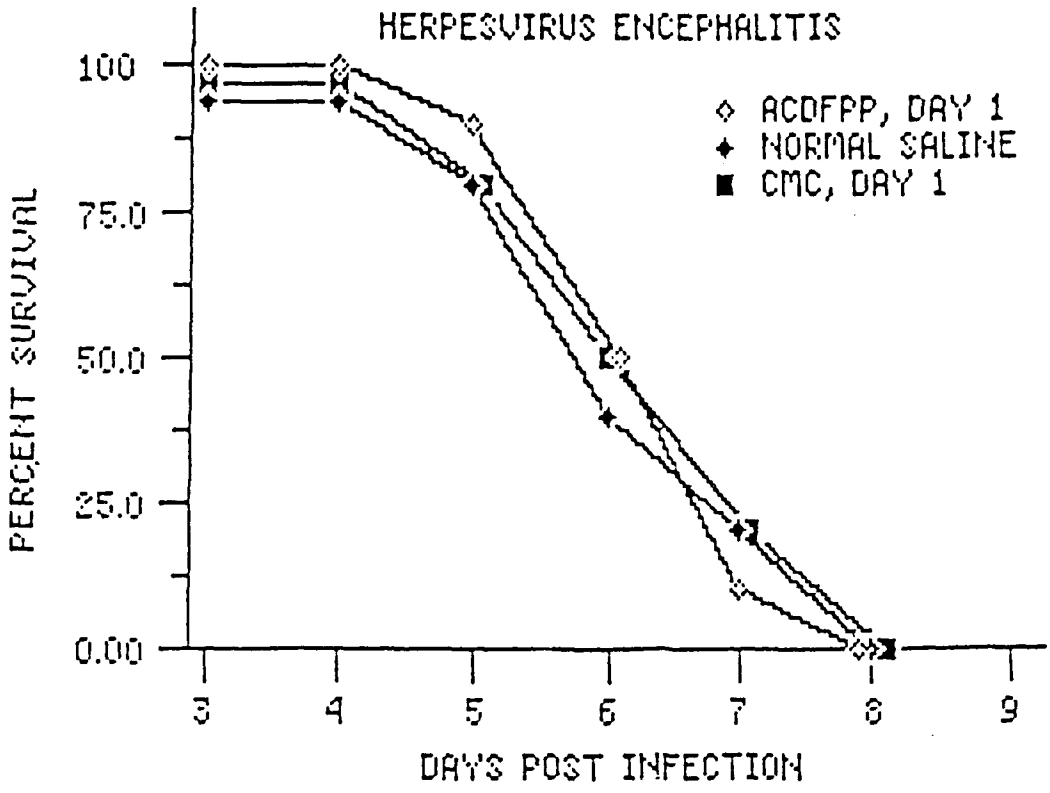
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	5.97	-
ACDFPP Day 0	6.44	NS
Saline Control	6.32	NS

Figure 72. Effect of ACDFPP, given on day 0, on resistance to herpesvirus-induced encephalitis.

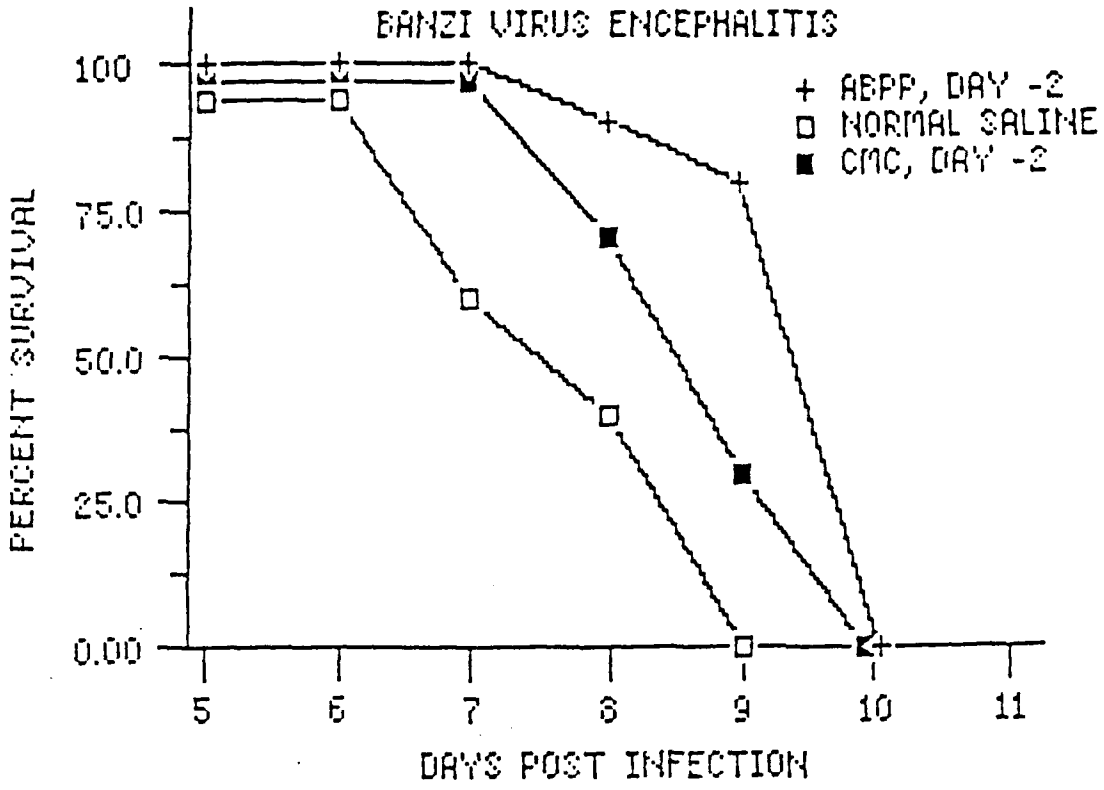
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	6.42	-
ACDFPP Day +1	6.45	NS
Saline Control	6.32	NS

Figure 73. Effect of ACDFPP, given on day +1, on resistance to herpesvirus-induced encephalitis.

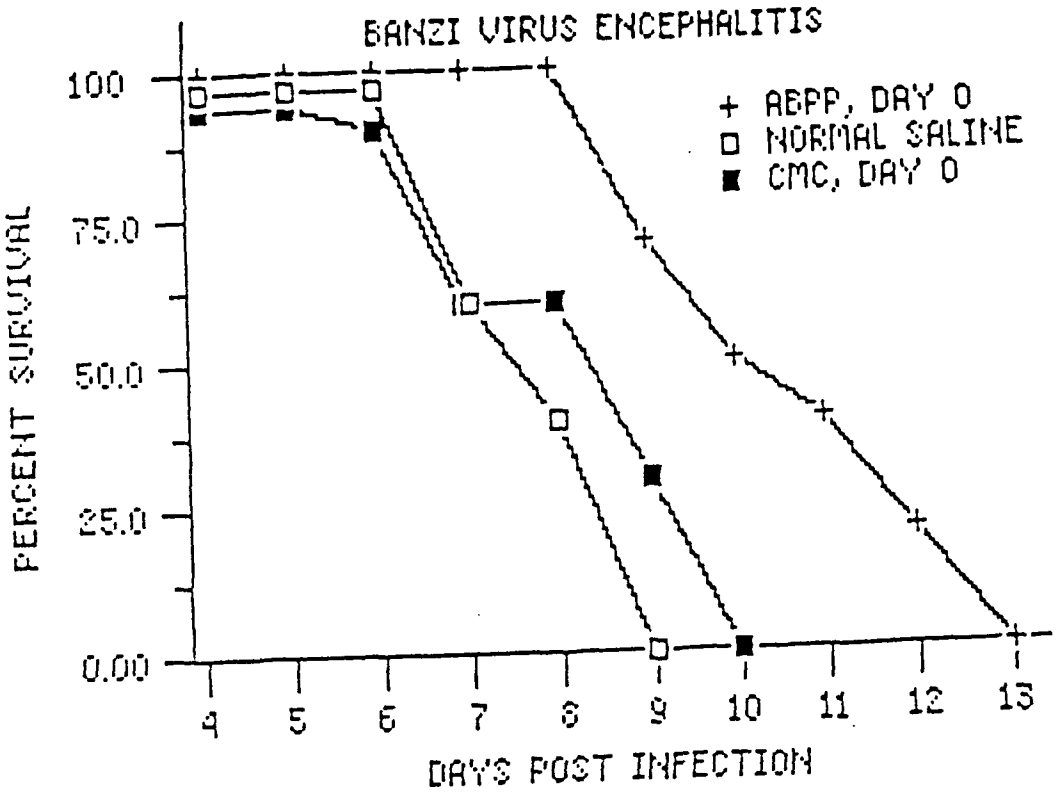
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) footpad challenge with 10 LD_{50} of HSV-1 (MB Strain). Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.97	-
ABPP Day -2	9.68	NS
Saline Control	7.95	<0.025

Figure 74. Effect of ABPP, given on day -2, on resistance to banzivirus-induced encephalitis.

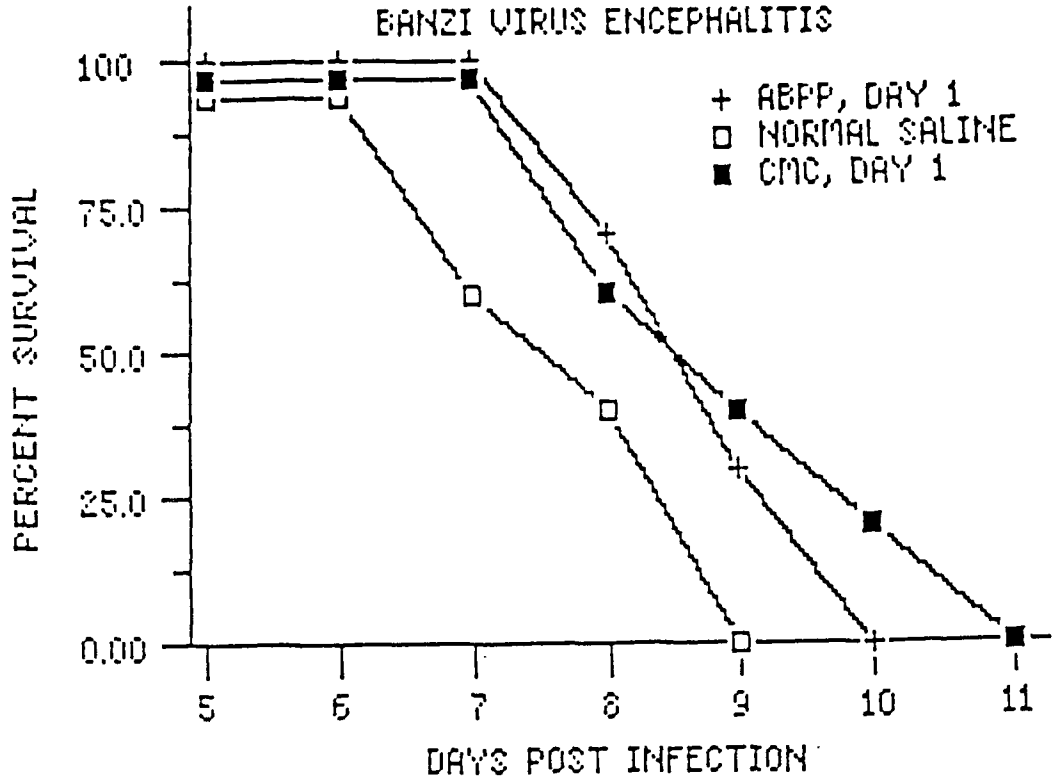
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.27	-
ABPP Day 0	10.69	<0.005
Saline Control	7.95	NS

Figure 75. Effect of ABPP, given on day 0, on resistance to banzivirus-induced encephalitis.

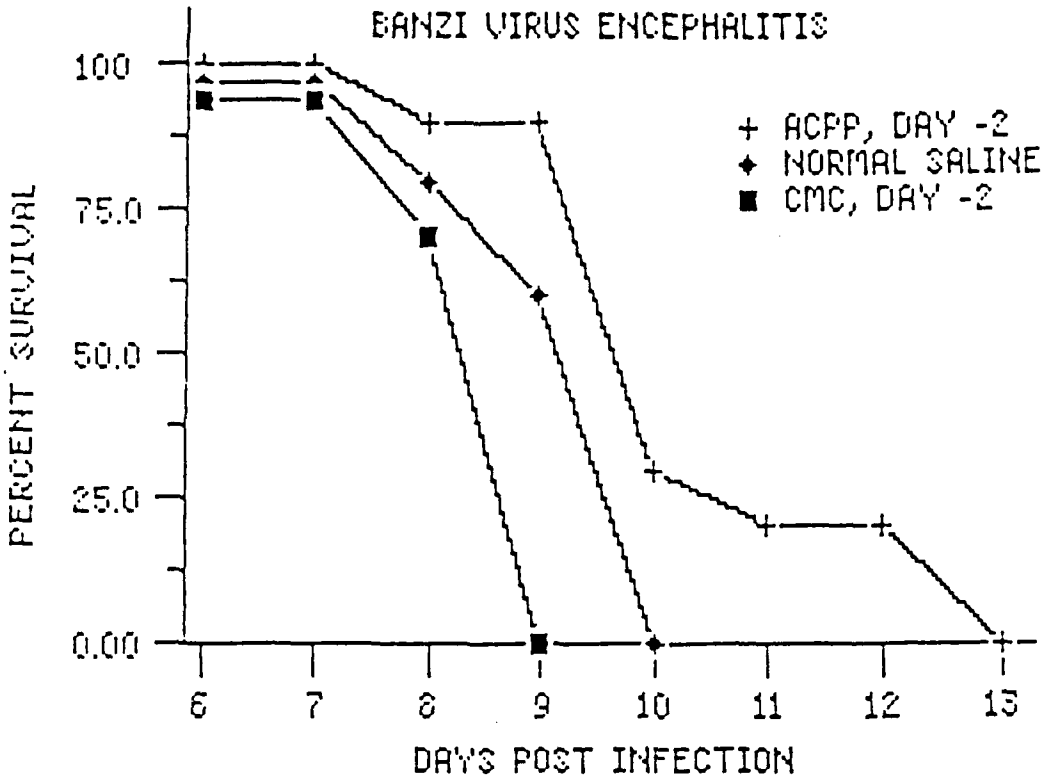
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. *NS = Not Significant.*



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	9.13	-
ABPP Day +1	8.97	NS
Saline Control	7.95	<0.025

Figure 76. Effect of ABPP, given on day +1, on resistance to banzivirus-induced encephalitis.

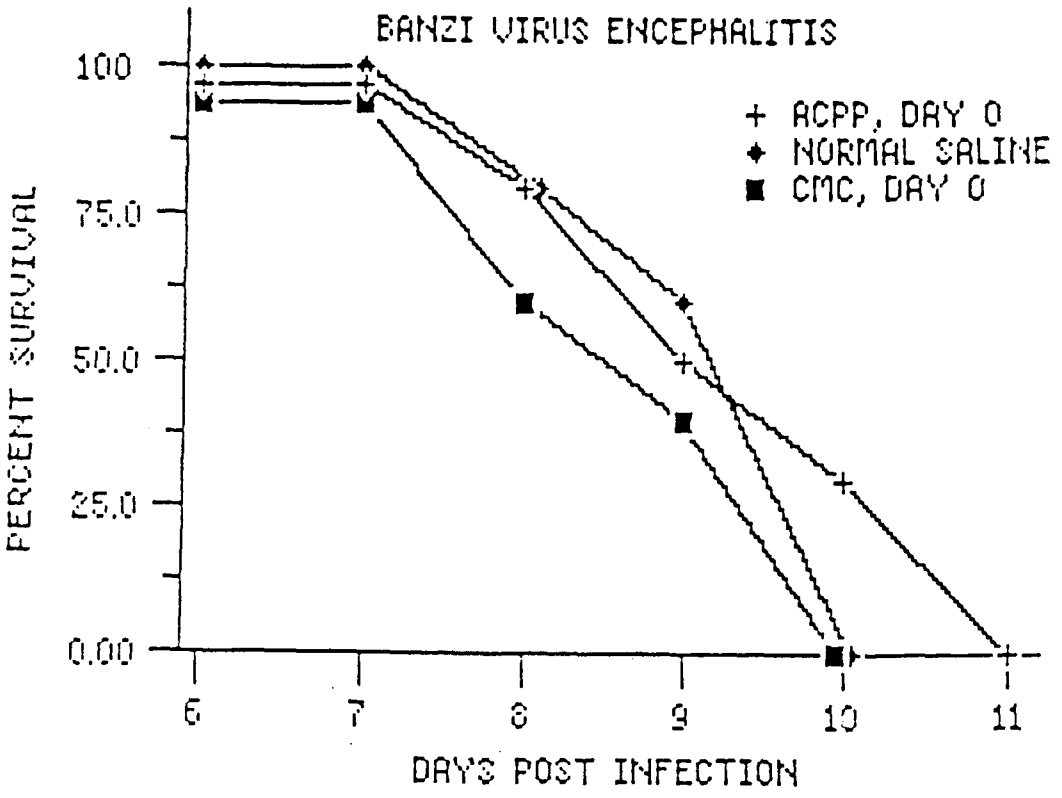
Mice were given ABPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.69	-
ACPP Day -2	10.40	<0.005
Saline Control	9.36	<0.05

Figure 77. Effect of ACPP, given on day -2, on resistance to banzivirus-induced encephalitis.

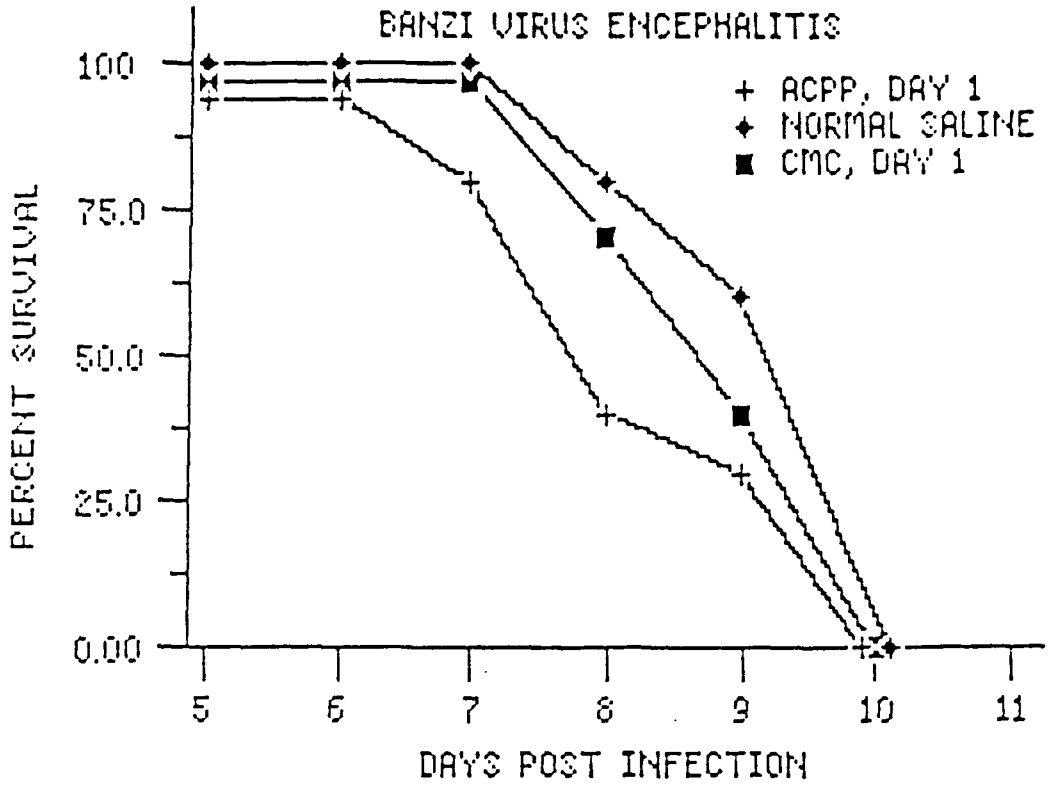
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.96	-
ACPP Day 0	9.53	NS
Saline Control	9.36	NS

Figure 78. Effect of ACPP, given on day 0, on resistance to banzivirus-induced encephalitis.

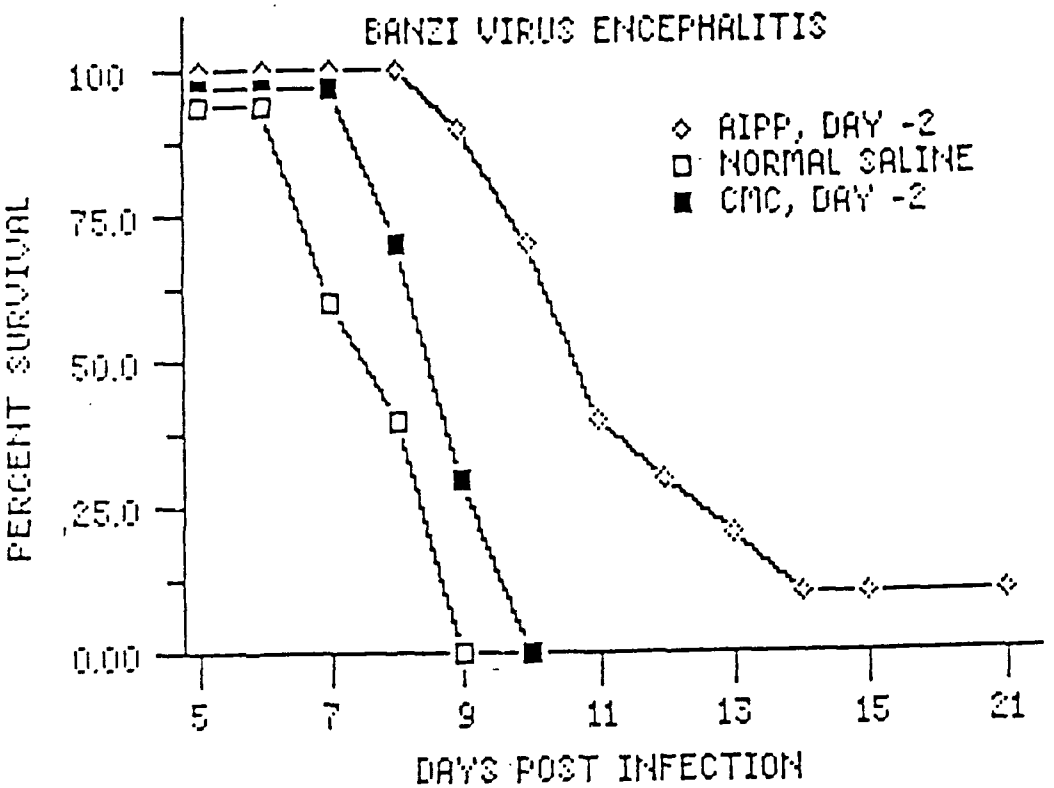
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	9.06	-
ACPP Day +1	8.43	NS
Saline Control	9.36	NS

Figure 79. Effect of ACPP, given on day +1, on resistance to banzivirus-induced encephalitis.

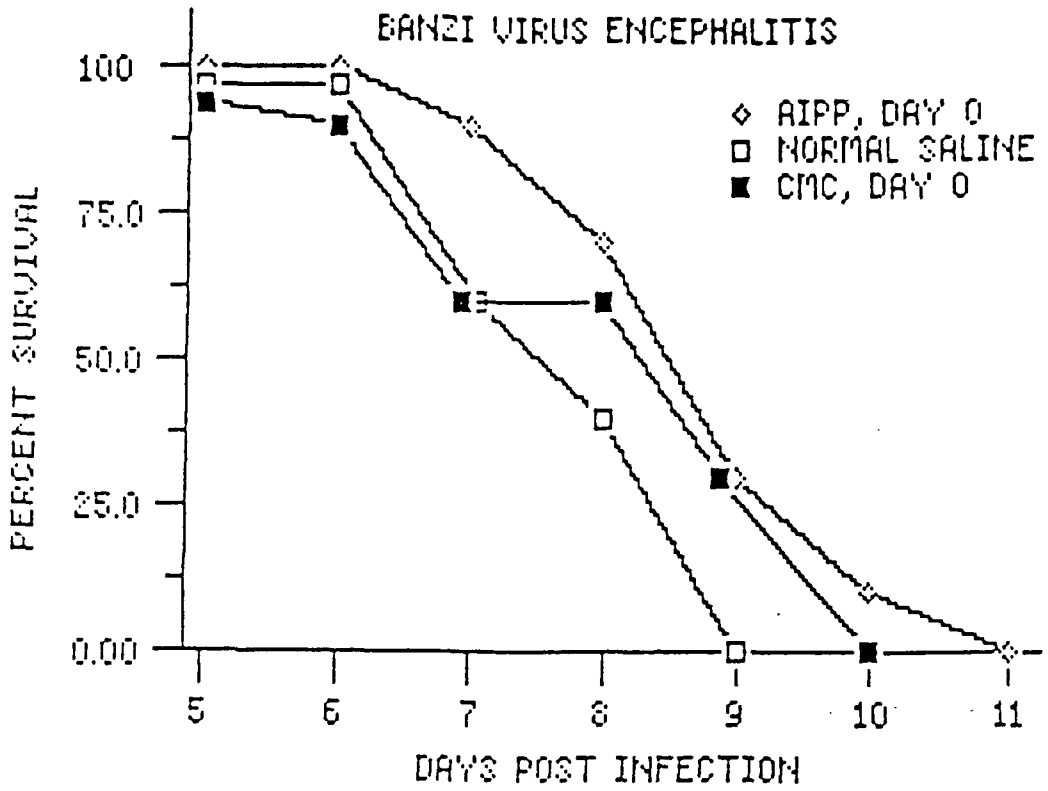
Mice were given ACPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.97	-
AIPP Day -2	11.13	<0.001
Saline Control	7.95	<0.025

Figure 80. Effect of AIPP, given on day -2, on resistance to banzivirus-induced encephalitis.

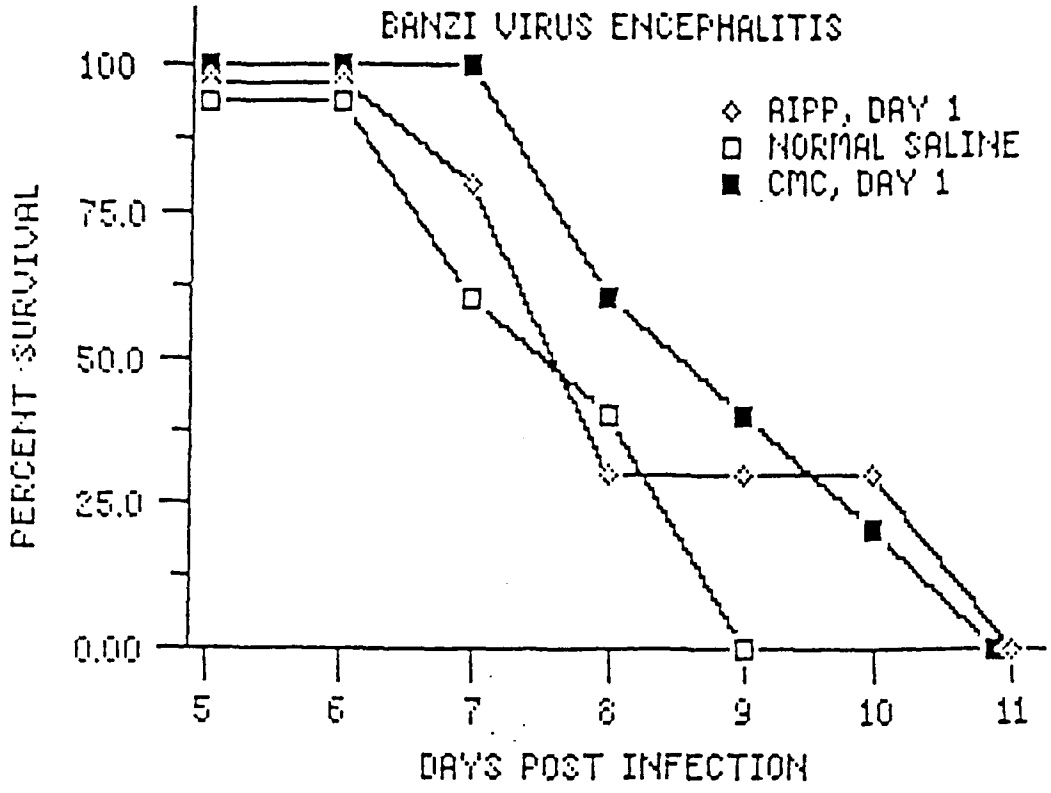
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.27	-
AIPP Day 0	8.93	NS
Saline Control	7.95	NS

Figure 81. Effect of AIPP, given on day 0, on resistance to banzivirus-induced encephalitis.

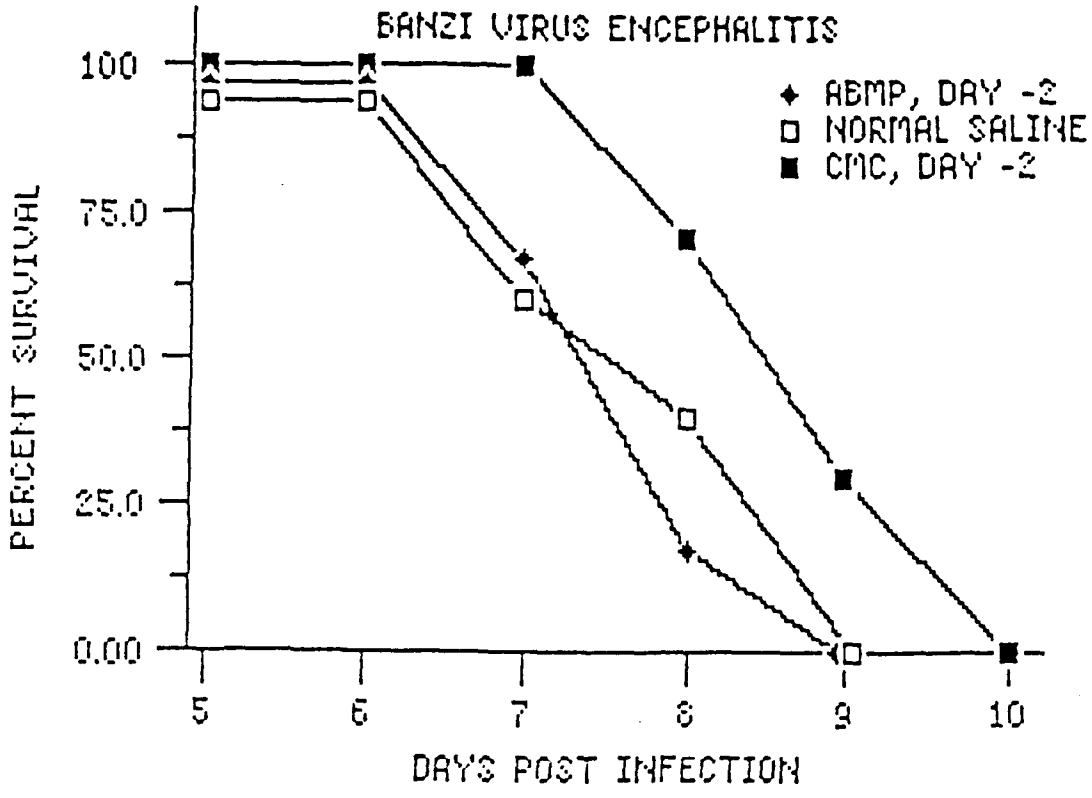
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	9.13	-
AIPP Day +1	8.57	NS
Saline Control	7.95	<0.025

Figure 82. *Effect of AIPP, given on day +1, on resistance to banzivirus-induced encephalitis.*

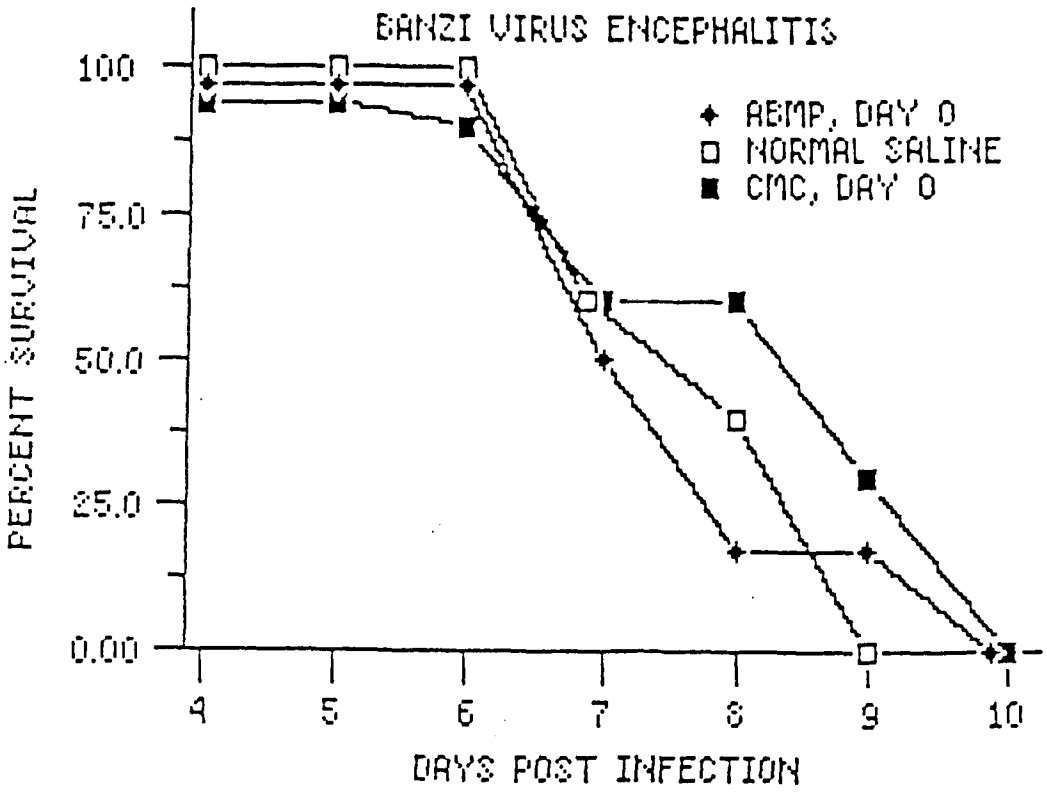
Mice were given AIPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.97	-
ABMP Day -2	7.80	<0.02
Saline Control	7.95	<0.025

Figure 83. Effect of ABMP, given on day -2, on resistance to banzivirus-induced encephalitis.

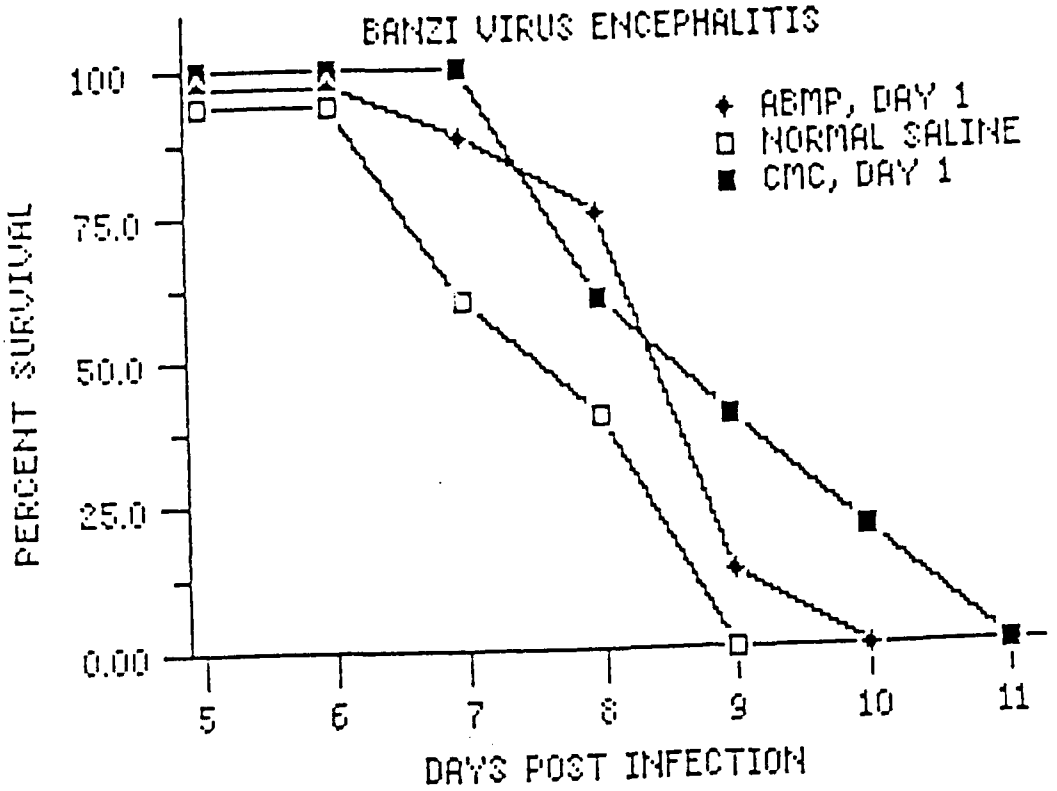
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.27	-
ABMP Day 0	7.77	NS
Saline Control	7.95	NS

Figure 84. Effect of ABMP, given on day 0, on resistance to banzivirus-induced encephalitis.

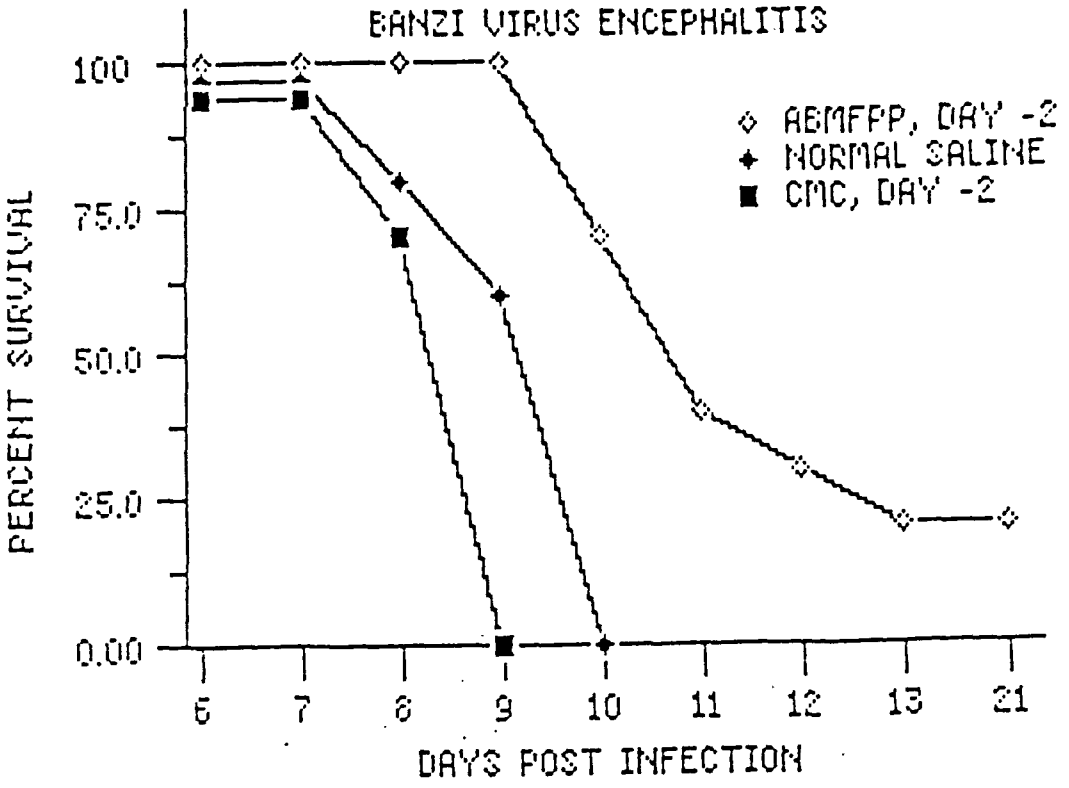
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	9.13	-
ABMP Day +1	8.71	NS
Saline Control	7.95	<0.025

Figure 85. Effect of ABMP, given on day +1, on resistance to banzivirus-induced encephalitis.

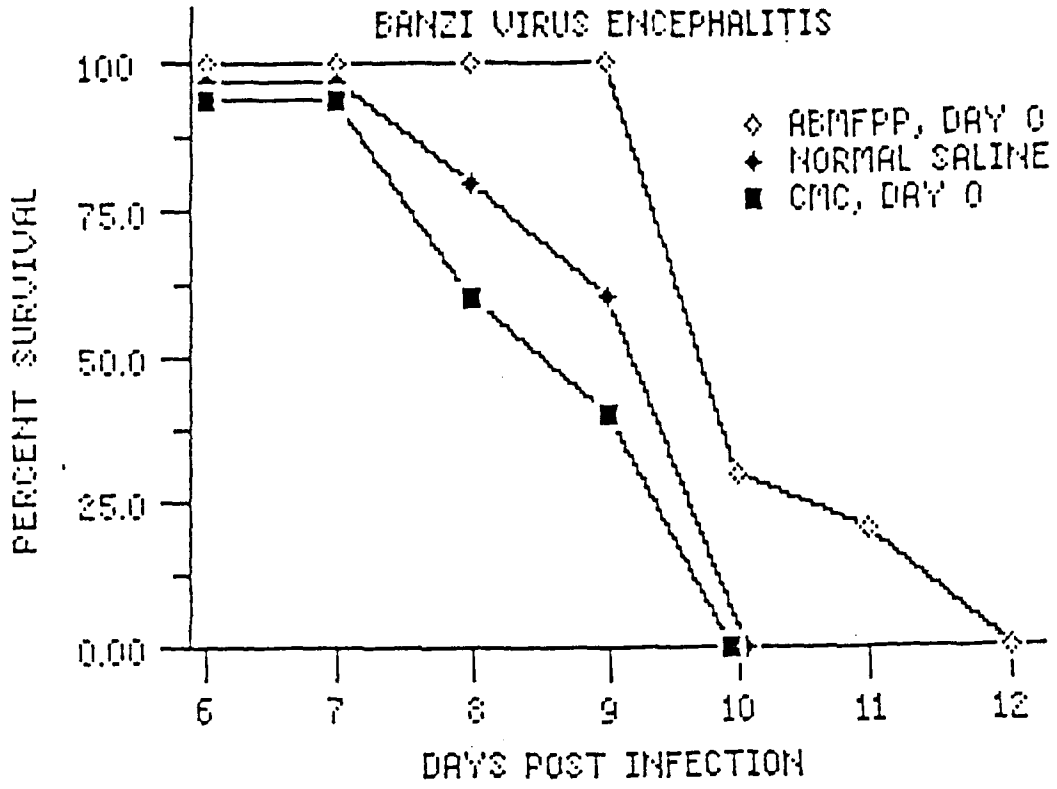
Mice were given ABMP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.69	-
ABMFPP Day -2	10.96	<0.001
Saline Control	9.36	<0.05

Figure 86. Effect of ABMFPP, given on day -2, on resistance to banzivirus-induced encephalitis.

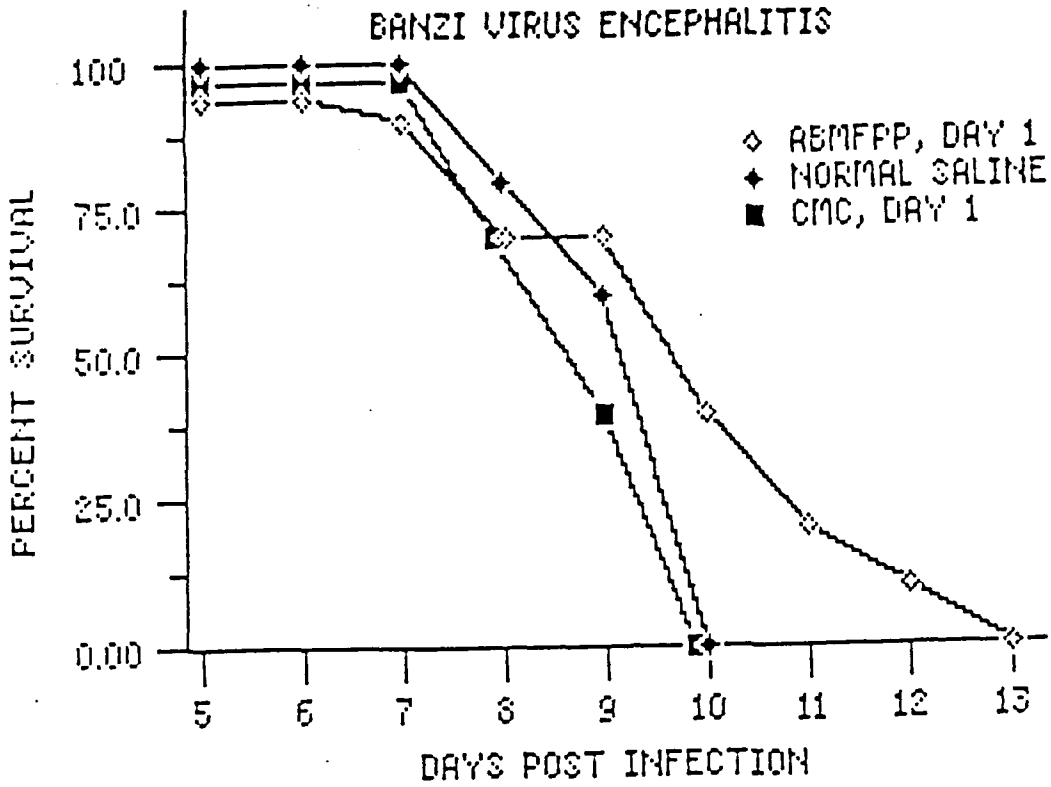
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.96	-
ABMFPP Day 0	10.47	<0.005
Saline Control	9.36	NS

Figure 87. Effect of ABMFPP, given on day 0, on resistance to banzivirus-induced encephalitis.

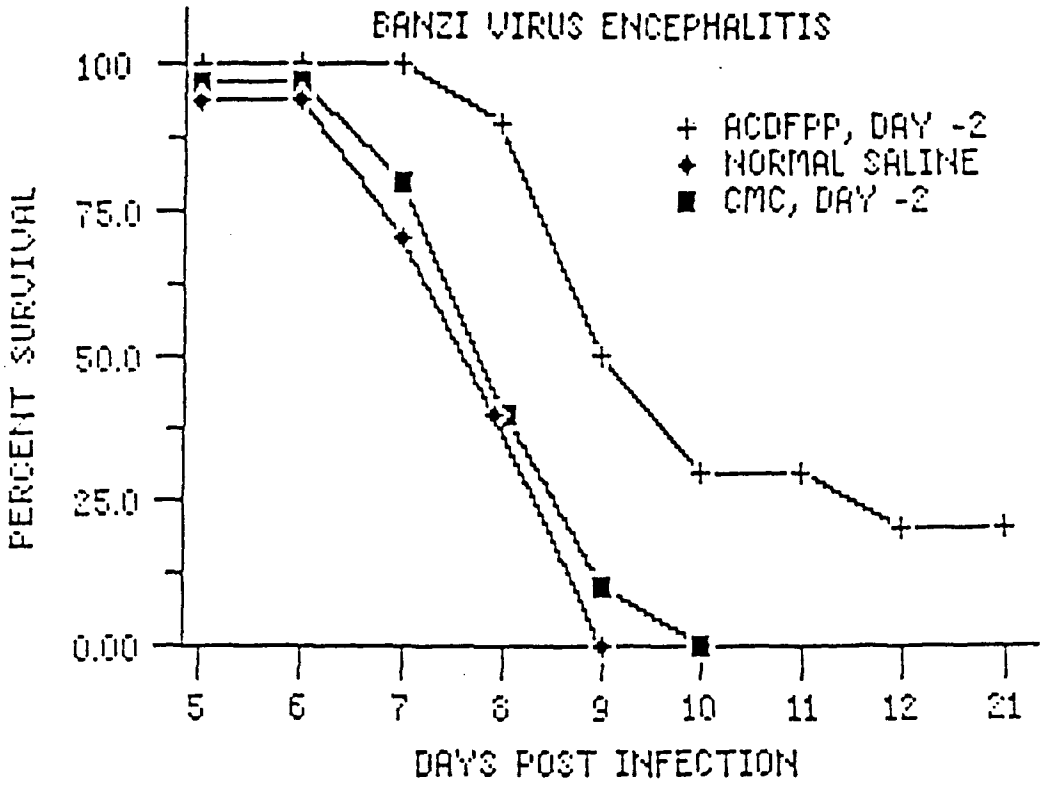
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	9.06	-
ABMFPP Day +1	9.83	NS
Saline Control	9.36	NS

Figure 88. Effect of ABMFPP, given on day +1, on resistance to banzivirus-induced encephalitis.

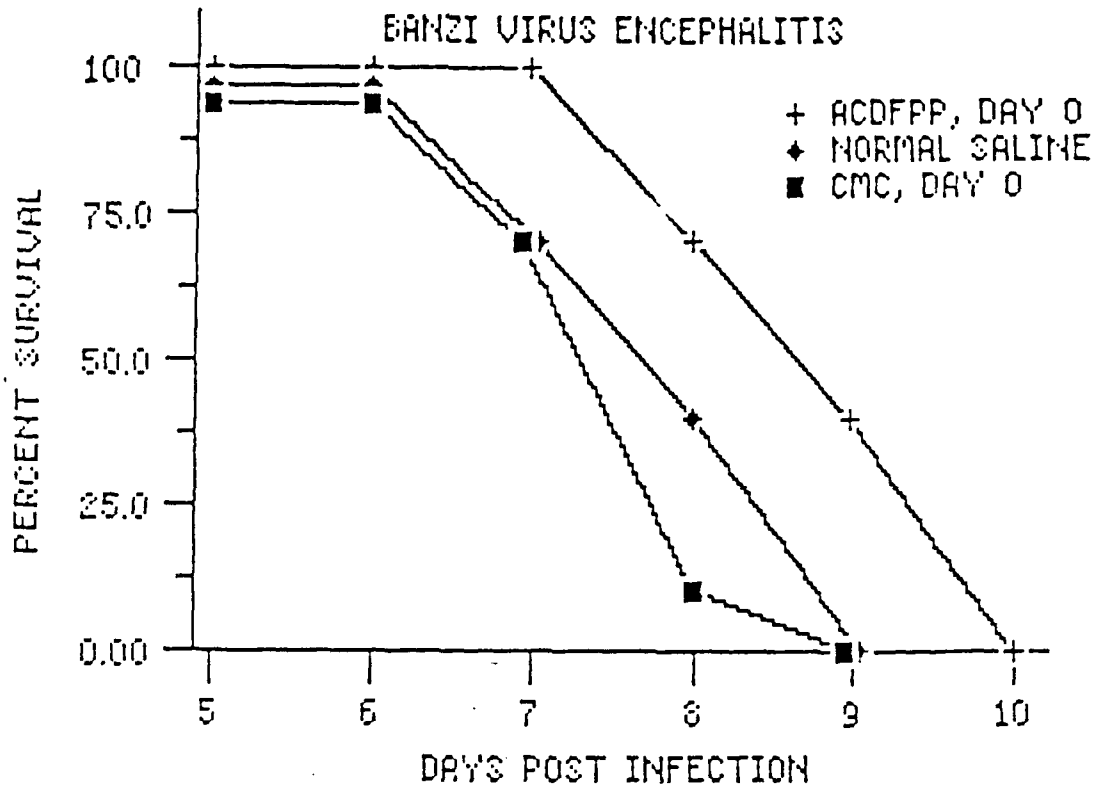
Mice were given ABMFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.25	-
ACDFPP Day -2	9.44	<0.05
Saline Control	8.06	NS

Figure 89. Effect of ACDFPP, given on day -2, on resistance to banzivirus-induced encephalitis.

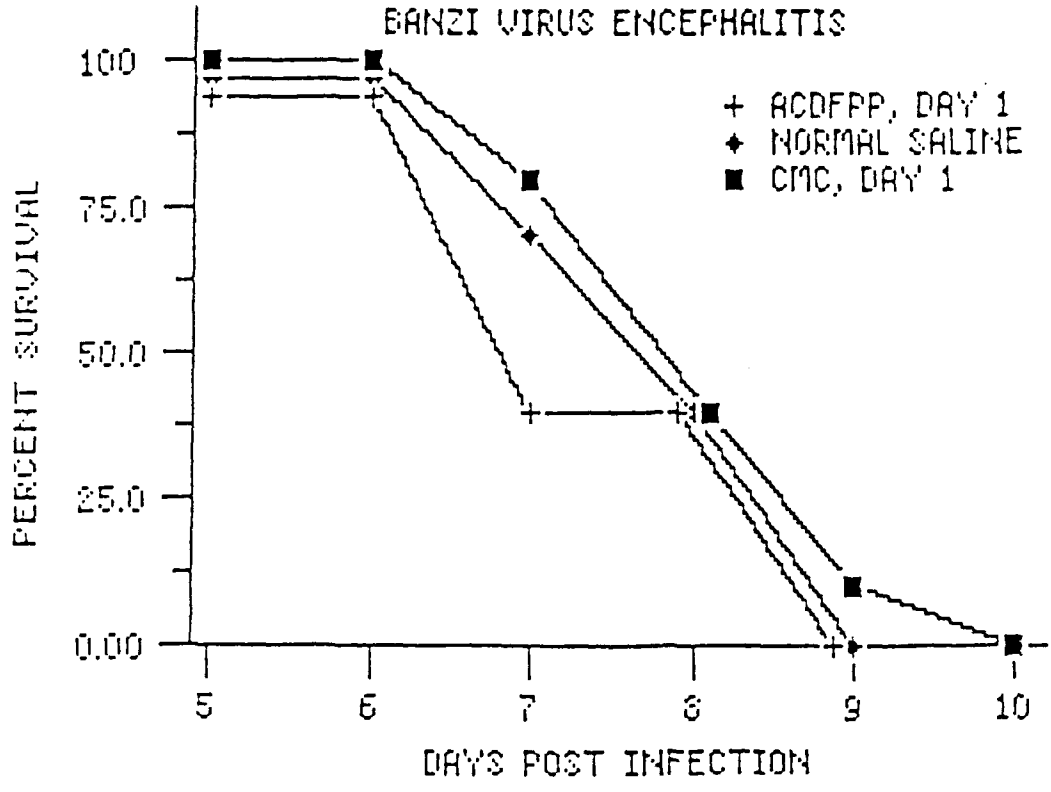
Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., two days prior to (D -2) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D -2. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	7.80	-
ACDFPP Day 0	9.07	<0.005
Saline Control	8.06	NS

Figure 90. Effect of ACDFPP, given on day 0, on resistance to banzivirus-induced encephalitis.

Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., on the day of (D 0) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D 0. A saline control group was also included. NS = Not Significant.



Treatment	Geometric Mean Survival Time (Days)	p Value
CMC Control	8.25	-
ACDFPP Day +1	7.74	NS
Saline Control	8.06	NS

Figure 91. Effect of ACDFPP, given on day +1, on resistance to banzivirus-induced encephalitis.

Mice were given ACDFPP (250 mg/kg) in 1% carboxymethyl cellulose (CMC), ip., one day after (D +1) subcutaneous challenge with 10 LD_{50} of banzivirus. Control animals received 1% CMC on D +1. A saline control group was also included. NS = Not Significant.

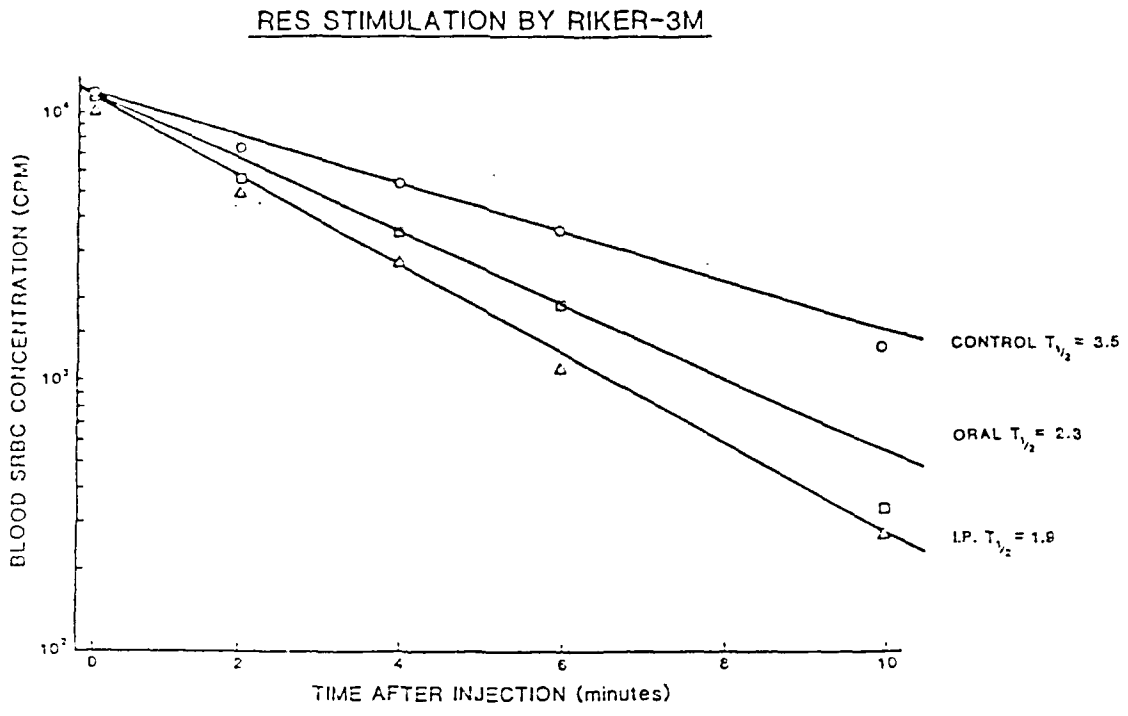
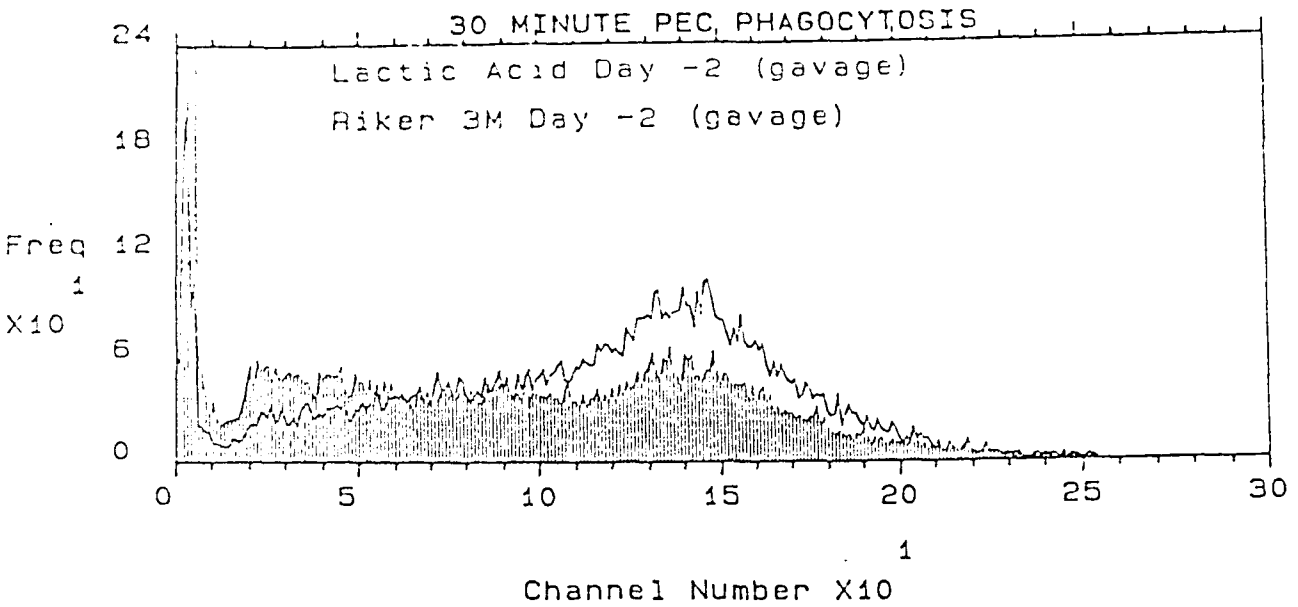


Figure 92. Reticuloendothelial system stimulation by Riker-3M compound S26308 (R-837). 10 mg/kg drug was administered in 0.2 ml 1% lactic acid orally or intraperitoneally 2 days before test for clearance of ^{51}Cr-labeled sheep erythrocytes (SRBC) from circulation. Control mice received the same volume of 1% lactic acid. The blood concentration of SRBC (counts per minute due to ^{51}Cr) was plotted against time after its injection and time to clear half of the injected material ($t_{1/2}$) was calculated from the line of best fit.

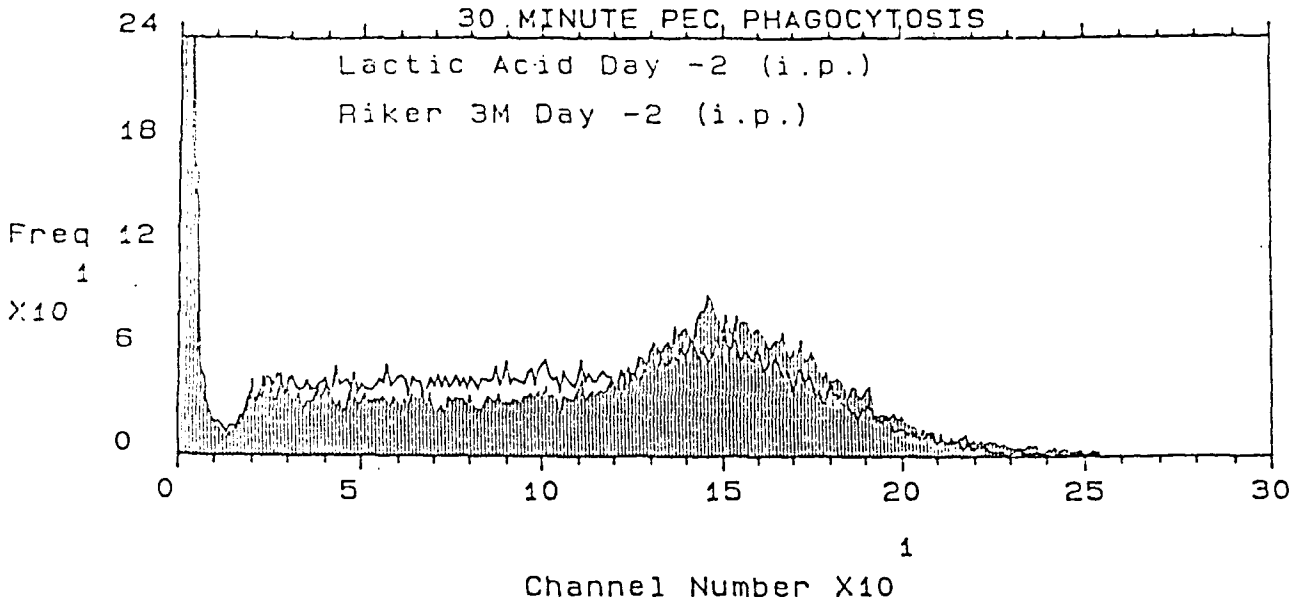


Treatment		% Phagocytic Cells			
		Total	Log 1	Log 2	Log 3
	Channel No.	61-255	61-85	86-171	172-255
1% Lactic Acid		55	9	37	8
Riker 3M in 1% Lactic Acid		79*	10	57*	12

Figure 93. Phagocytosis by peritoneal exudate cells following oral administration of Riker 3M on day -2.

Mice were given Riker 3M (10 mg/kg), orally, two days prior to assaying for phagocytosis of fluoresceinated *S. aureus* by flow cytometry. The x axis in the figure represents log fluorescence intensity plotted on a three cycle scale and the y axis represents number of cells. The total number of phagocytic cells and the percentages in logs 1, 2 and 3 were determined by integrating the areas under the curve. The fluorescence in channels 1-60 represents primarily autofluorescence. The shaded curve is the data for the control.

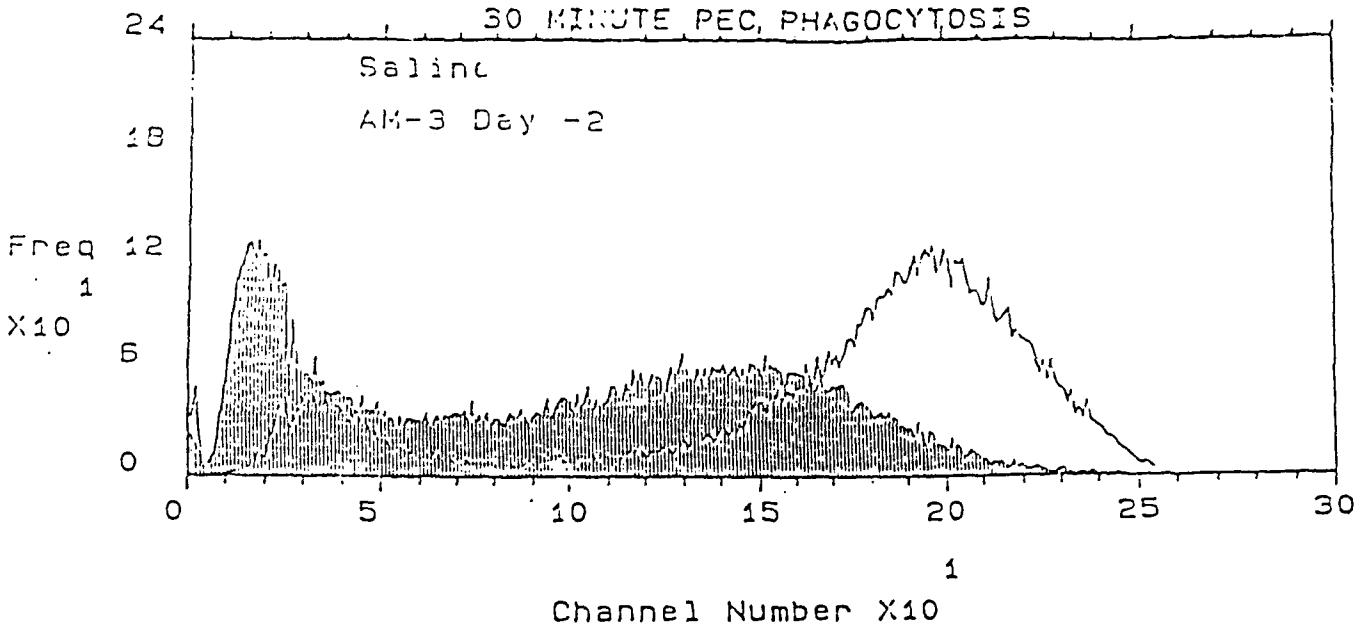
* $p < 0.01$



Treatment	% Phagocytic Cells			
	Total	Log 1	Log 2	Log 3
Channel No.	61-255	61-85	86-171	172-255
1% Lactic Acid	71	8	47	17
Riker 3M in 1% Lactic Acid	66	10	47	9

Figure 94. Phagocytosis by peritoneal exudate cells following intraperitoneal administration of Riker 3M on day -2.

Mice were given Riker 3M (10 mg/kg), intraperitoneally, two days prior to assaying for phagocytosis of fluoresceinated *S. aureus* by flow cytometry. The x axis in the figure represents log fluorescence intensity plotted on a three cycle scale and the y axis represents number of cells. The total number of phagocytic cells and the percentages in logs 1, 2 and 3 were determined by integrating the areas under the curve. The fluorescence in channels 1-60 represents primarily autofluorescence. The shaded curve is the data for the control.

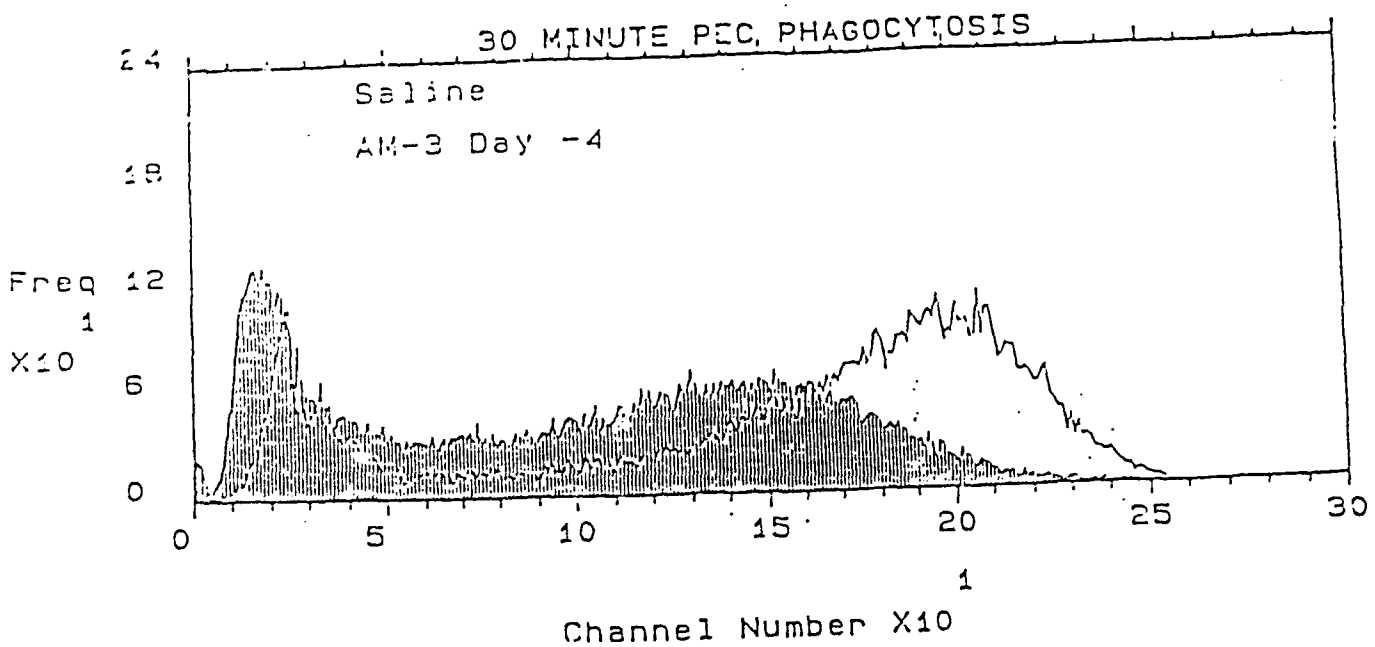


Treatment	% Phagocytic Cells			
	Total	Log 1	Log 2	Log 3
Channel No.	61-255	61-85	86-171	172-255
Control	64	8	44	13
AM-3	86*	2*	21*	63*

Figure 95. Phagocytosis by peritoneal exudate cells following intraperitoneal administration of AM-3 on day -2.

Mice were given AM-3 (400 mg/kg), intraperitoneally, two days prior to assaying for phagocytosis of fluoresceinated *S. aureus* by flow cytometry. The x axis in the figure represents log fluorescence intensity plotted on a three cycle scale and the y axis represents number of cells. The total number of phagocytic cells and the percentages in logs 1, 2 and 3 were determined by integrating the areas under the curve. The fluorescence in channels 1-60 represents primarily autofluorescence. The shaded curve is the data for the control.

* $p < 0.01$

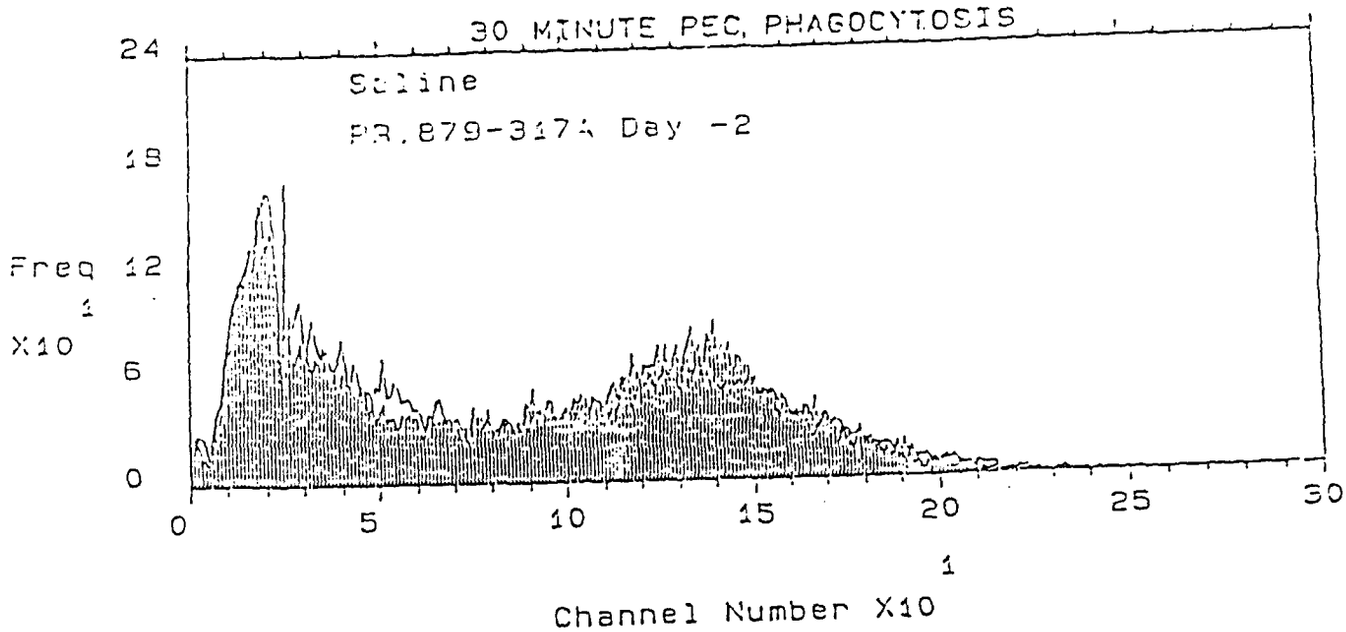


Treatment	% Phagocytic Cells			
	Total	Log 1	Log 2	Log 3
Channel No.	61-255	61-85	86-171	172-255
Control	64	8	44	13
AM-3	81*	3*	28*	50*

Figure 96. Phagocytosis by peritoneal exudate cells following intraperitoneal administration of AM-3 on day -4.

Mice were given AM-3 (400 mg/kg), intraperitoneally, four days prior to assaying for phagocytosis of fluoresceinated *S. aureus* by flow cytometry. The x axis in the figure represents log fluorescence intensity plotted on a three cycle scale and the y axis represents number of cells. The total number of phagocytic cells and the percentages in logs 1, 2 and 3 were determined by integrating the areas under the curve. The fluorescence in channels 1-60 represents primarily autofluorescence. The shaded curve is the data for the control.

* $p < 0.01$

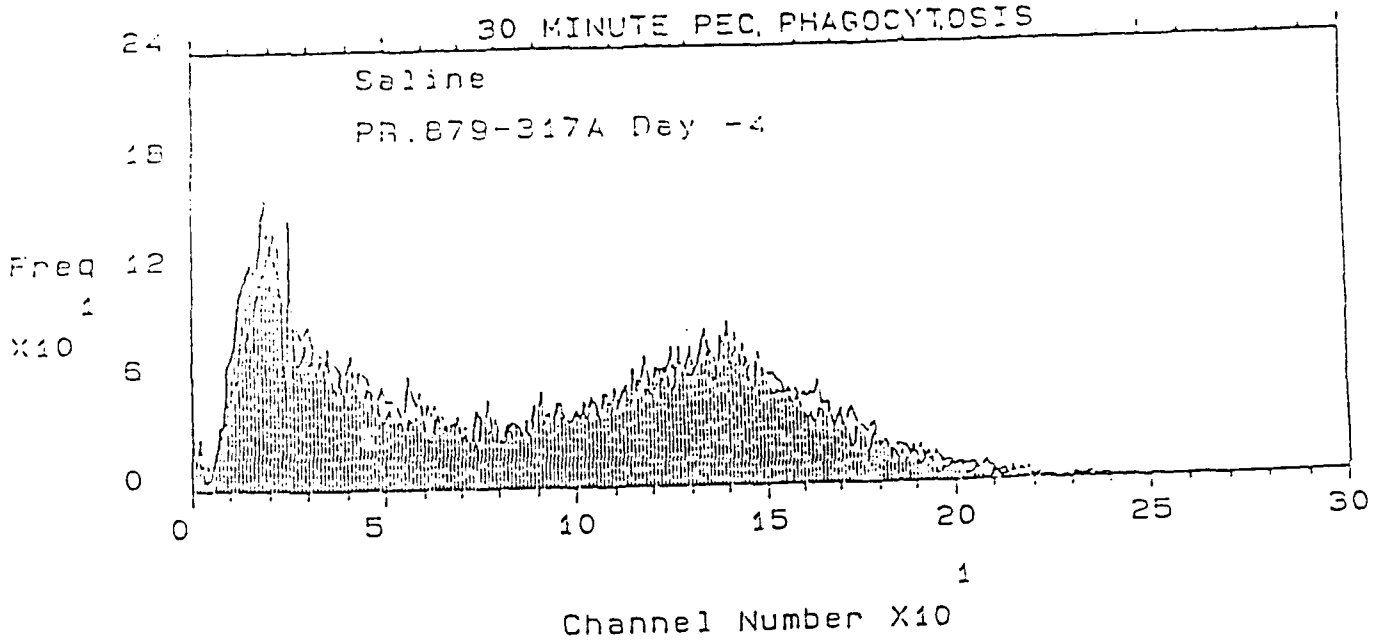


Treatment	% Phagocytic Cells			
	Total	Log 1	Log 2	Log 3
Channel No.	61-255	61-85	86-171	172-255
Control	57	7	44	6
PR 879-317A	52	8	37	7

Figure 97. Phagocytosis by peritoneal exudate cells following intraperitoneal administration of PR 879-317A on day -2.

Mice were given PR 879-317A (50 mg/kg), intraperitoneally, two days prior to assaying for phagocytosis of fluoresceinated *S. aureus* by flow cytometry. The x axis in the figure represents log fluorescence intensity plotted on a three cycle scale and the y axis represents number of cells. The total number of phagocytic cells and the percentages in logs 1, 2 and 3 were determined by integrating the areas under the curve. The fluorescence in channels 1-60 represents primarily autofluorescence. The shaded curve is the data for the control.

* $p < 0.01$

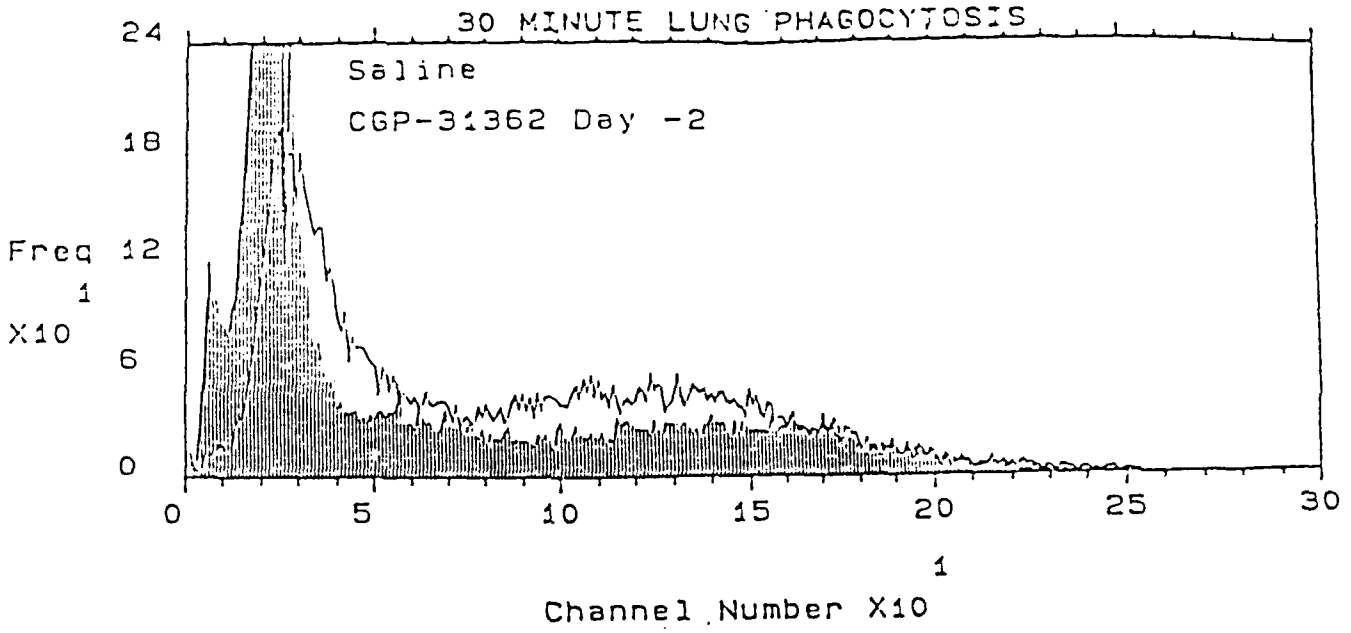


Treatment		% Phagocytic Cells			
		Total	Log 1	Log 2	Log 3
	Channel No.	61-255	61-85	86-171	172-255
Control		57	7	44	6
PR 879-317A		60	8	44	8

Figure 98. Phagocytosis by peritoneal exudate cells following intraperitoneal administration of PR 879-317A on day -4.

Mice were given PR 879-317A (50 mg/kg), intraperitoneally, four days prior to assaying for phagocytosis of fluoresceinated *S. aureus* by flow cytometry. The x axis in the figure represents log fluorescence intensity plotted on a three cycle scale and the y axis represents number of cells. The total number of phagocytic cells and the percentages in logs 1, 2 and 3 were determined by integrating the areas under the curve. The fluorescence in channels 1-60 represents primarily autofluorescence. The shaded curve is the data for the control.

* $p < 0.01$

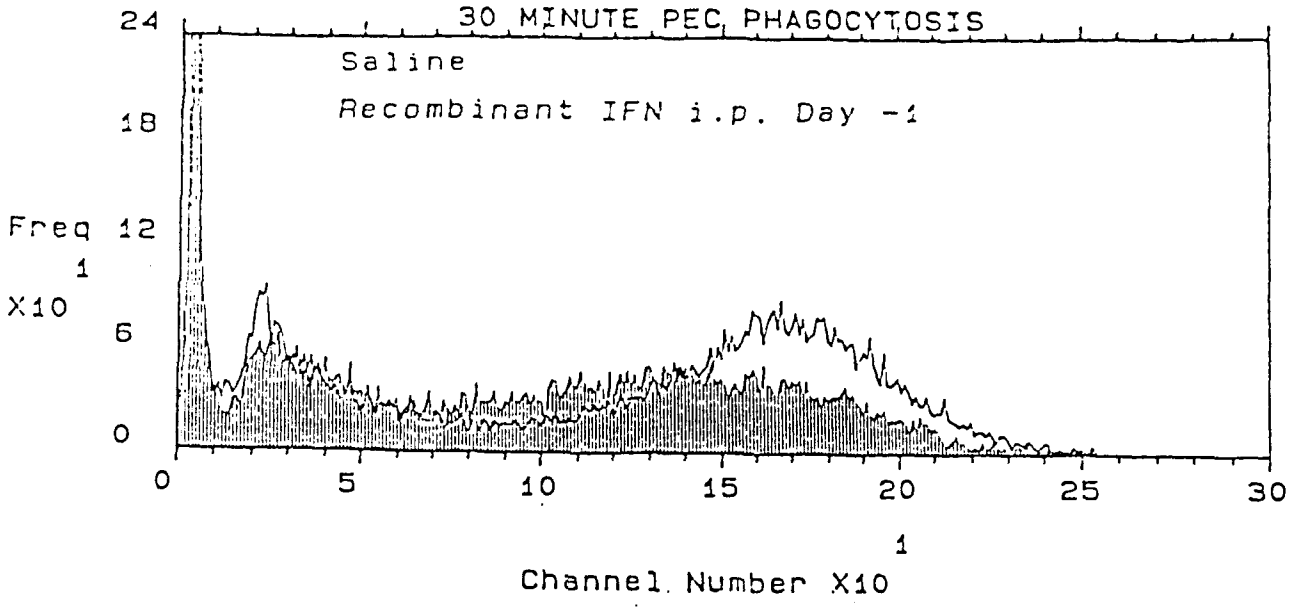


Treatment	% Phagocytic Cells			
	Total	Log 1	Log 2	Log 3
Channel No.	61-255	61-85	86-171	172-255
Control	34	6	21	7
CGP 31,362	52*	9	35*	8

Figure 99. Phagocytosis by lung cells following treatment on day -2 with CGP 31,362.

Mice were given CGP 31,362 (100 μg/kg), intranasally, two days prior to assaying for phagocytosis of fluoresceinated *S. aureus* by flow cytometry. The x axis in the figure represents log fluorescence intensity plotted on a three cycle scale and the y axis represents number of cells. The total number of phagocytic cells and the percentages in logs 1, 2 and 3 were determined by integrating the areas under the curve. The fluorescence in channels 1-60 represents primarily autofluorescence. The shaded curve is the data for the control.

* $p < 0.01$

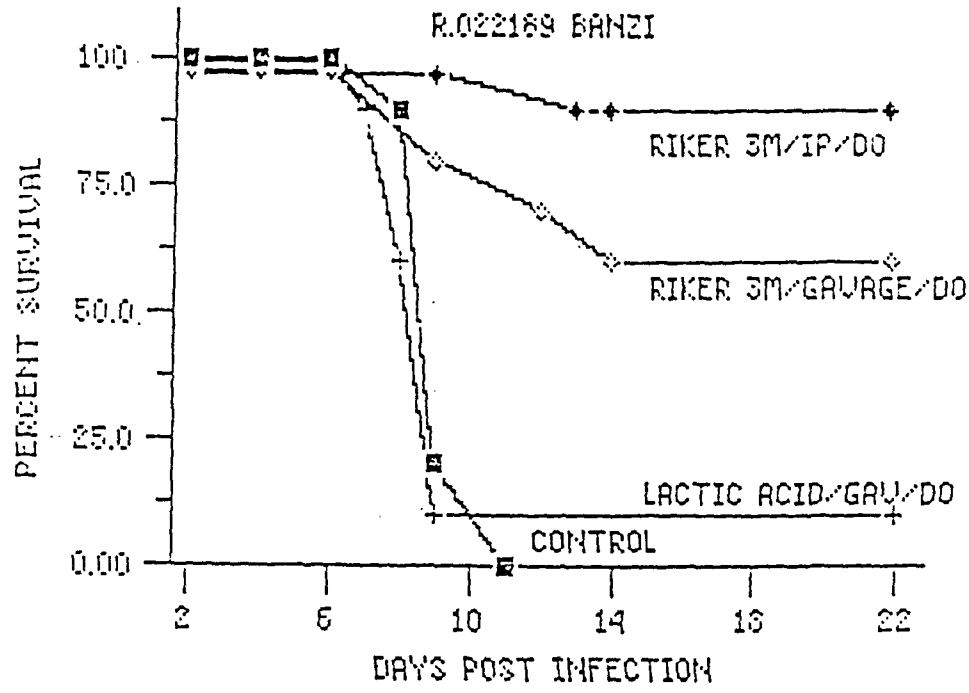


Treatment	% Phagocytic Cells			
	Total	Log 1	Log 2	Log 3
Channel No.	61-255	61-85	86-171	172-255
Control	52	7	33	12
Recombinant IFN (ip)	65	5	35	25*

Figure 100. Phagocytosis by peritoneal exudate cells following intraperitoneal administration of recombinant interferon on day -1.

Mice were given recombinant interferon ($1X10^6$ IU), intraperitoneally, one day prior to assaying for phagocytosis of fluoresceinated *S. aureus* by flow cytometry. The x axis in the figure represents log fluorescence intensity plotted on a three cycle scale and the y axis represents number of cells. The total number of phagocytic cells and the percentages in logs 1, 2 and 3 were determined by integrating the areas under the curve. The fluorescence in channels 1-60 represents primarily autofluorescence. The shaded curve is the data for the control.

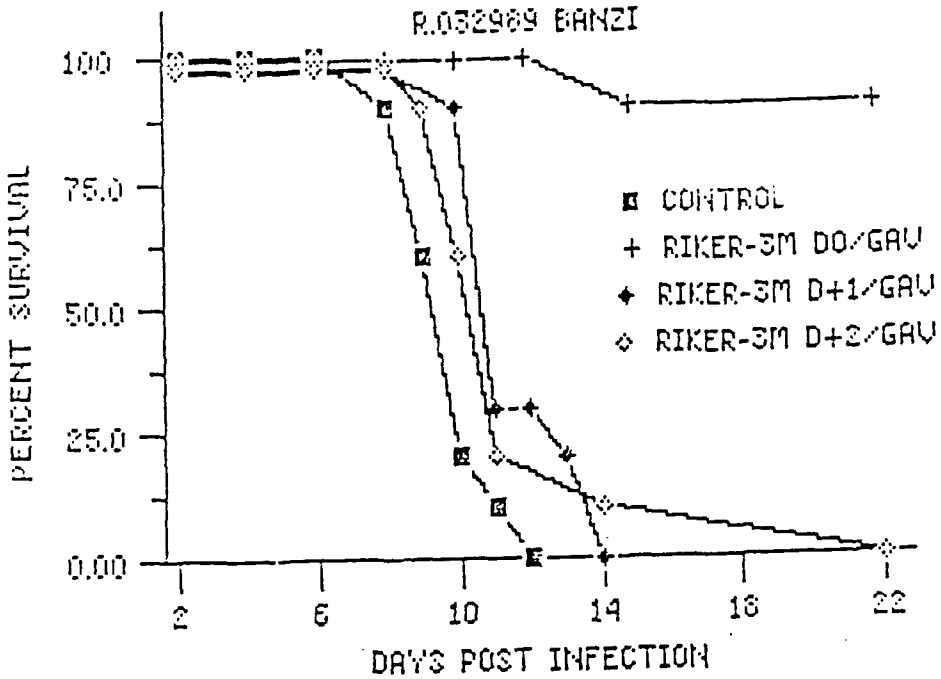
* $p < 0.01$



Treatment	Geometric Mean Survival Time (days)	p Value
Riker 3M		
oral, day		
0	16.1	<0.001
i.p., day		
0	20.0	<0.001
Control, oral (1% lactic acid)	9.2	---
Control, i.p. (1% lactic acid)	9.3	---

Figure 101. Effect of intraperitoneal or oral administration of Riker 3M (10 mg/kg) on Banzi virus-induced encephalitis.

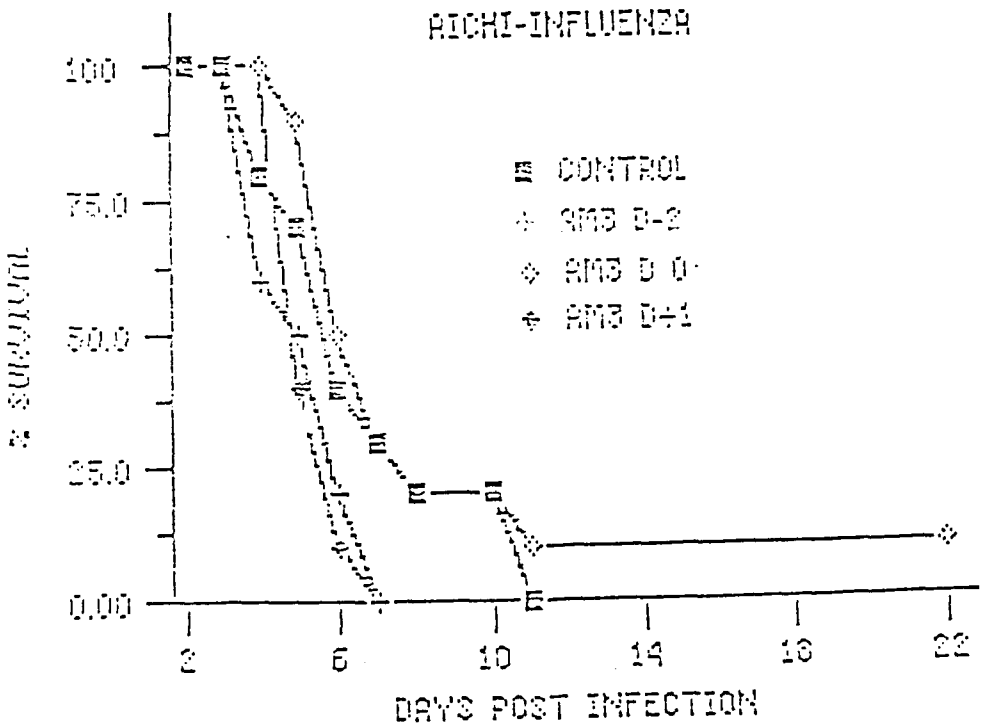
Mice were given a single dose of Riker 3M (10 mg/kg) on the day indicated and challenged subcutaneously with 1 LD_{80} of Banzi virus. Ten mice per treatment group were examined using the Wilcoxon rank analysis.



Treatment	Geometric Mean Survival Time (days)	p Value
Riker 3M (10 mg/kg) day		
0	21.2	<0.001
+1	11.6	NS
+2	11.5	NS
Control (1% lactic acid)	9.4	---

Figure 102. Effects of orally administered Riker 3M on Banzi virus-induced encephalitis.

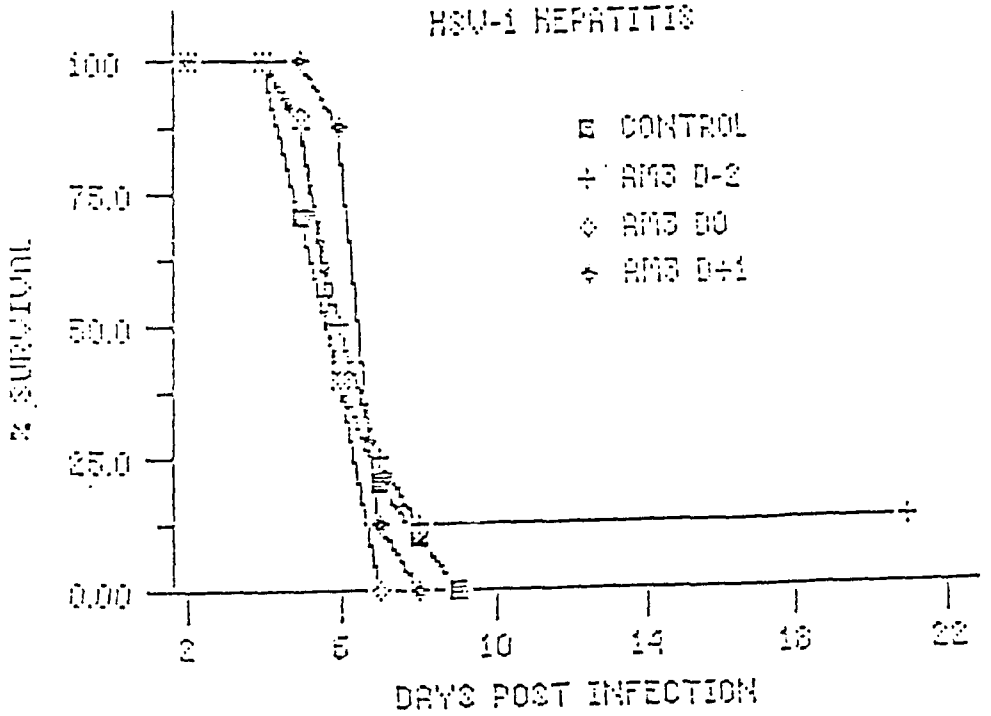
Mice were given a single dose of Riker 3M (10 mg/kg) on the day indicated and challenged subcutaneously with 1 LD_{80} of Banzi virus. Ten mice per treatment group were examined using the Wilcoxon rank analysis.



Treatment	Geometric Mean Survival Time (days)	p Value
AM-3		
day-2	5.2	NS
day 0	7.5	NS
day+1	5.5	NS
Saline Control	6.4	—

Figure 103. AM-3 in therapy of influenza.

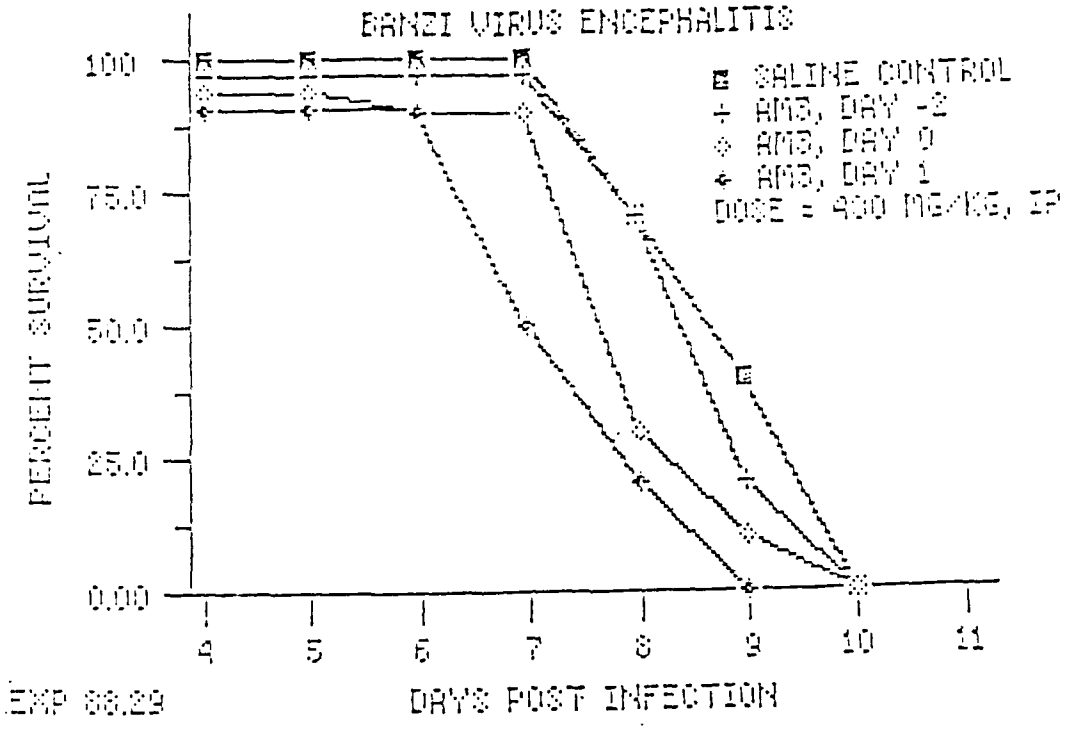
Mice were inoculated intraperitoneally with a single dose (400 mg/kg) of drug on the days indicated (day 0 is the day of infection). Virus (influenza, Aichi H3N2) was administered intravenously (0.05 ml = 10 LD50) and the mice examined daily for 21 days. Ten mice per treatment group were examined using Wilcoxon rank analysis.



Treatment	Geometric Mean Survival Time (days)	p Value
AM-3		
day-2	7.4	NS
day 0	6.3	NS
day+1	7.0	NS
Saline Control	6.3	—

Figure 104. AM-3 in therapy of HSV-1 induced hepatitis.

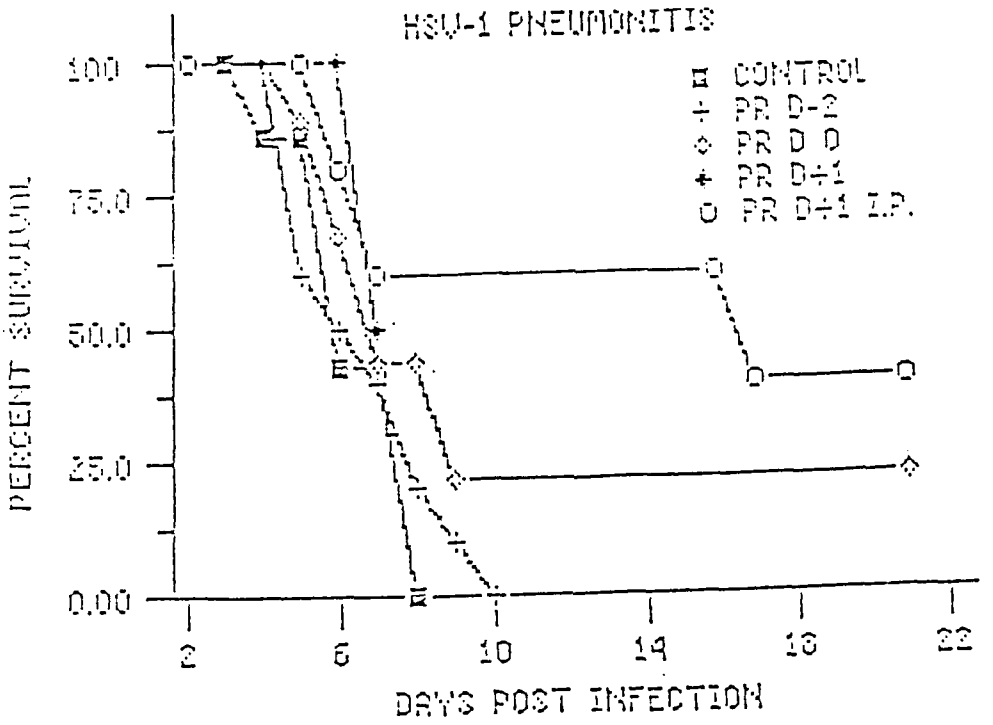
Mice were inoculated intraperitoneally with a single dose (400 mg/kg) of drug on the days indicated (day 0 is the day of infection). Virus (HSV-1, MB strain) was administered intravenously (0.2 ml = 10 LD50) and the mice examined daily for 21 days. Ten mice per treatment group were examined using Wilcoxon rank analysis.



Treatment	Geometric Mean Survival Time (days)	p Value
AM-3		
day-2	8.9	NS
day 0	8.2	<.05
day+1	7.6	<.005
Saline Control	9.1	—

Figure 105. AM-3 in therapy of Banzi encephalitis.

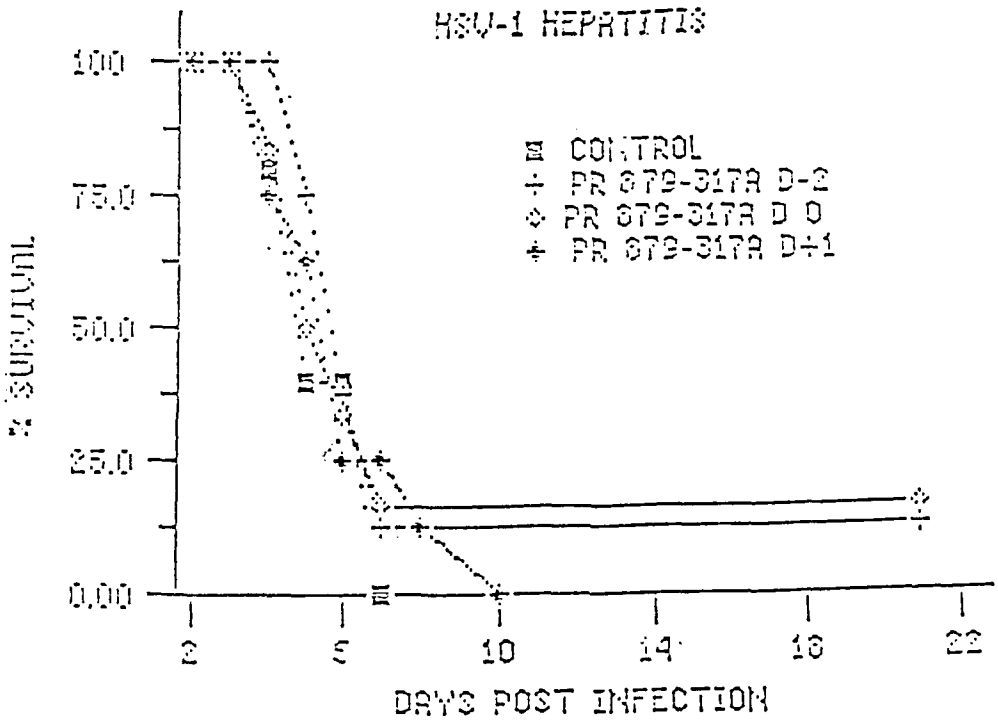
Mice were inoculated intraperitoneally with a single dose (400 mg/kg) of drug on the days indicated (day 0 is the day of infection). Banzi virus was administered subcutaneously (0.2 ml = 10 LD_{50}) and the mice examined daily for 21 days. Ten mice per treatment group were examined using Wilcoxon rank analysis.



Treatment	Geometric Mean Survival Time (days)	p Value
PR 879-317A		
day-2 (i.v.)	7.1	NS
day 0 (i.v.)	8.8	NS
day+1 (i.v.)	7.5	NS
day+1 (i.p.)	12.6	<.01
Saline Control	6.4	—

Figure 106. PR 879-317A in therapy of HSV-1 induced pneumonitis.

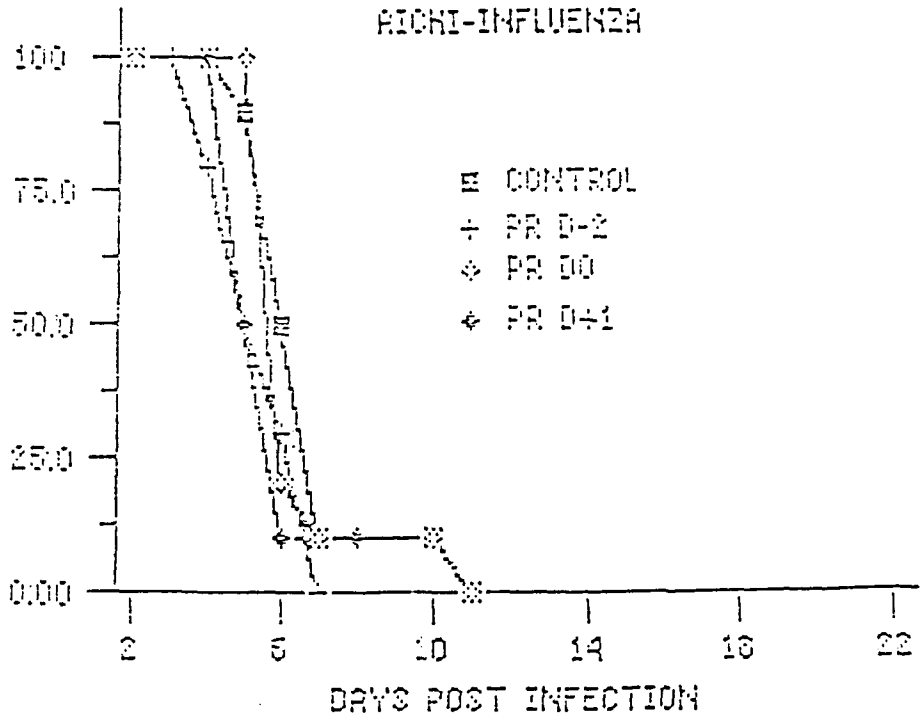
Mice were inoculated (i.v. or i.p.) with a single dose (10 mg/kg) of drug on the days indicated (day 0 is the day of infection). Virus (HSV-1, VR-3 strain) was administered intranasally (0.05 ml = 10 LD_{50}) and the mice examined daily for 21 days. Ten mice per treatment group were examined using Wilcoxon rank analysis.



Treatment	Geometric Mean Survival Time (days)	p Value
PR 879-317A		
day-2	7.0	NS
day 0	6.7	NS
day+1	5.9	NS
Saline Control	5.5	—

Figure 107. PR 879-317A in therapy of HSV-1 induced hepatitis.

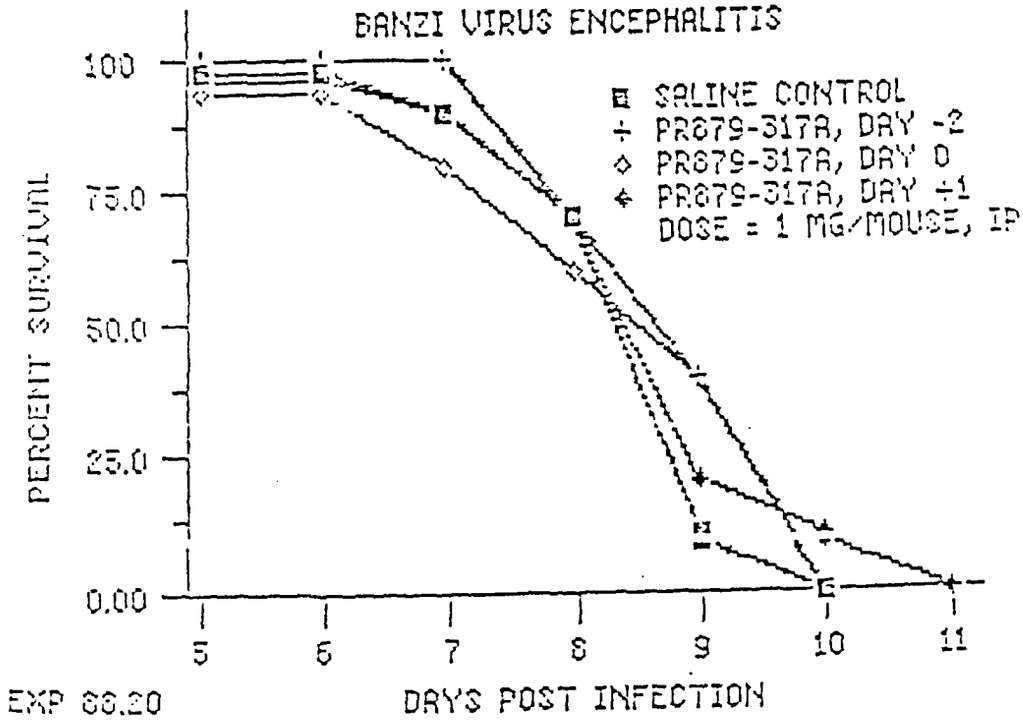
Mice were inoculated intraperitoneally with a single dose (50 mg/kg) of drug on the days indicated (day 0 is the day of infection). Virus (HSV-1, MB strain) was administered intravenously (0.2 ml = 10 LD_{50}) and the mice examined daily for 21 days. Ten mice per treatment group were examined using Wilcoxon rank analysis.



Treatment	Geometric Mean Survival Time (days)	p Value
PR 879-317A		
day-2	5.5	NS
day 0	6.4	NS
day+1	5.8	NS
Saline Control	6.7	—

Figure 108. PR 879-317A in therapy of influenza.

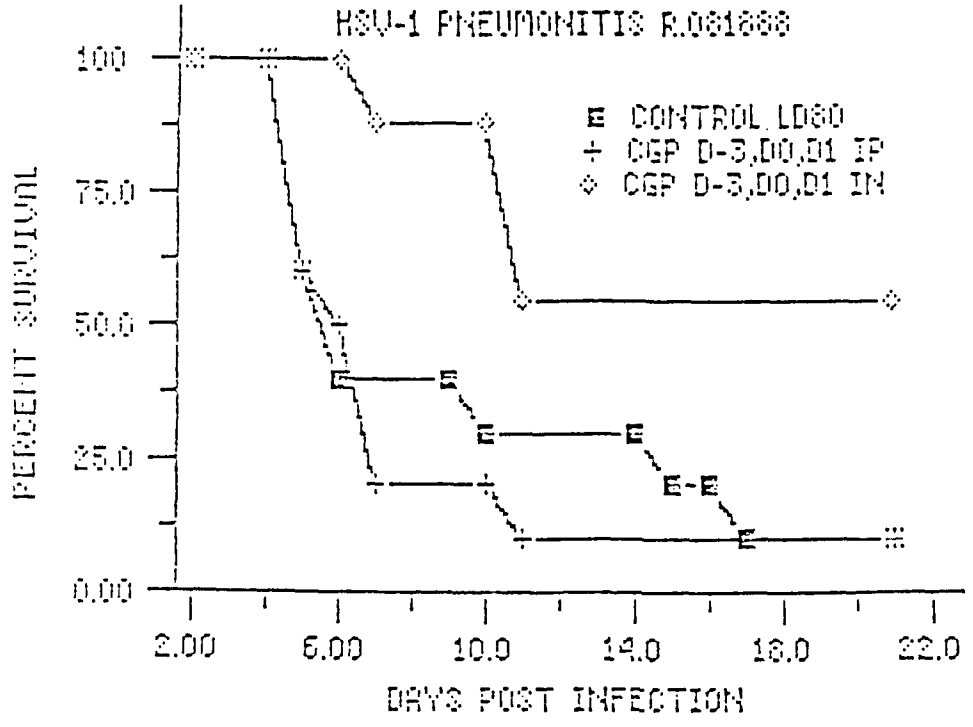
Mice were inoculated intraperitoneally with a single dose (50 mg/kg) of drug on the days indicated (day 0 is the day of infection). Virus (influenza, Aichi H3N2) was administered intravenously (0.05 ml = 10 LD_{50}) and the mice examined daily for 21 days. Ten mice per treatment group were examined using Wilcoxon rank analysis.



Treatment	Geometric Mean Survival Time (days)	p Value
PR 879-317A		
day-2	9.1	NS
day 0	8.8	NS
day+1	8.9	NS
Saline Control	8.7	—

Figure 109. PR 879-317A in therapy of Banzi encephalitis.

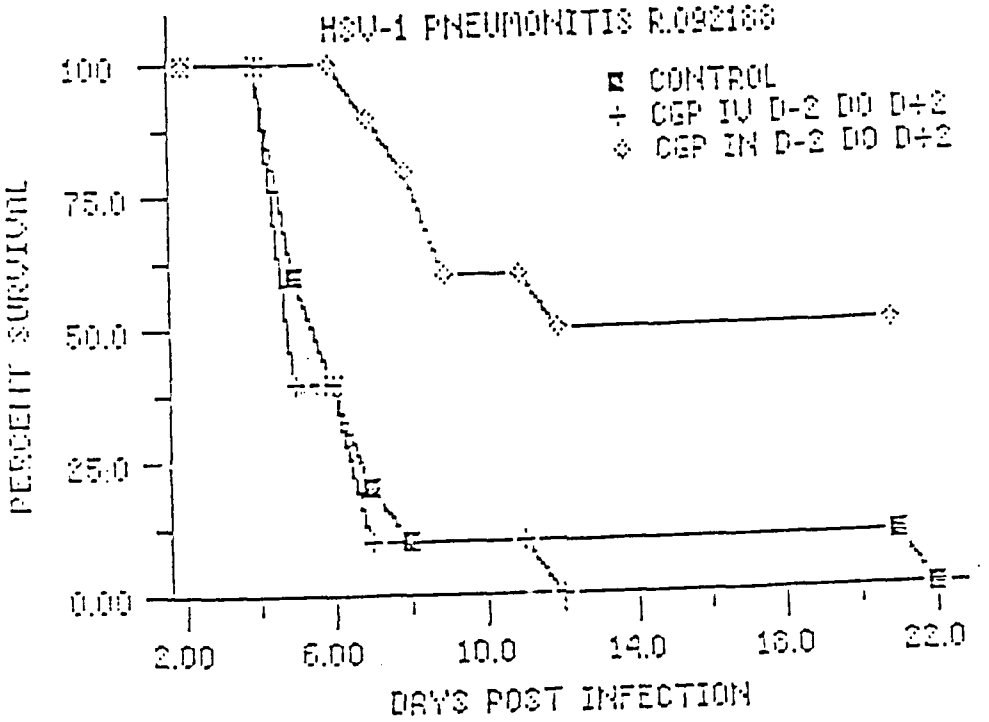
Mice were inoculated intraperitoneally with a single dose (50 mg/kg) of drug on the days indicated (day 0 is the day of infection). Banzi virus was administered subcutaneously (0.2 ml = 10 LD_{50}) and the mice examined daily for 21 days. Ten mice per treatment group were examined using Wilcoxon rank analysis.



Treatment	Geometric Mean Survival Time (Days)	p Value
CGP 31,362		
i.p. Days -3,0,+1	7.0	NS
i.n. Days -3,0,+1	15.0	0.02
Saline control	8.1	--

Figure 110. Intranasal versus intraperitoneal administration of CGP 31,362 in the therapy of HSV-1 pneumonitis.

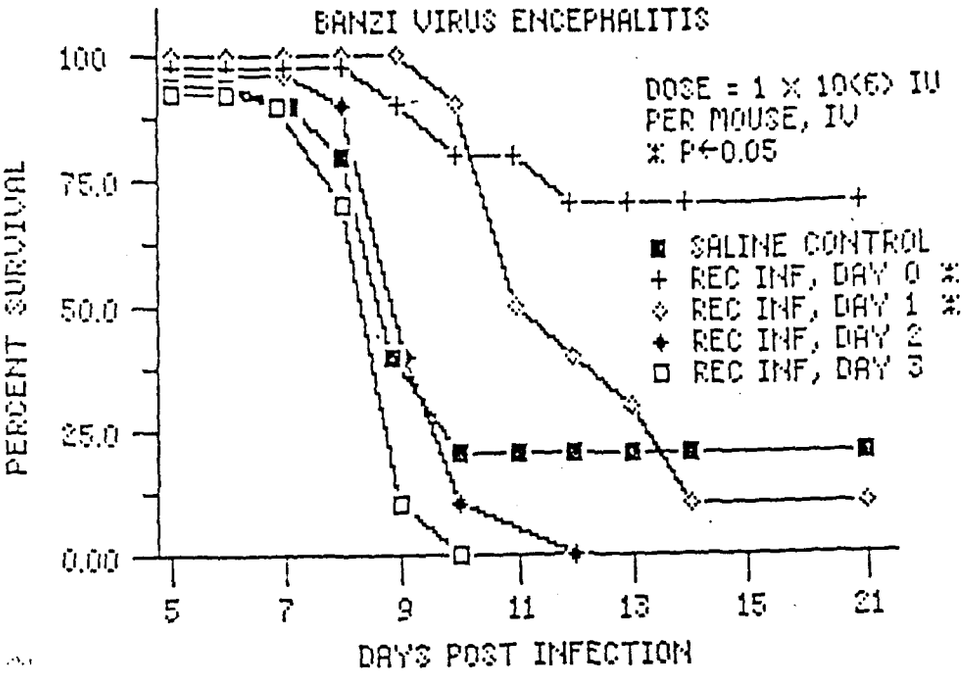
Mice were inoculated with a 100 μg/kg of CGP 31,362 on the days indicated. HSV-1 was administered intranasally to 4 week old male mice. Ten mice per treatment group were evaluated using Wilcoxon Rank Analysis.



Treatment	Geometric Mean Survival Time (Days)	p Value
CGP 31,362		
i.v. Days -2,0,+1	6.3	NS
i.n. Days -2,0,+1	13.6	0.005
Saline control	6.7	---

Figure 111. Intranasal versus intra venous administration of CGP 31,362 in the therapy of HSV-1 pneumonitis.

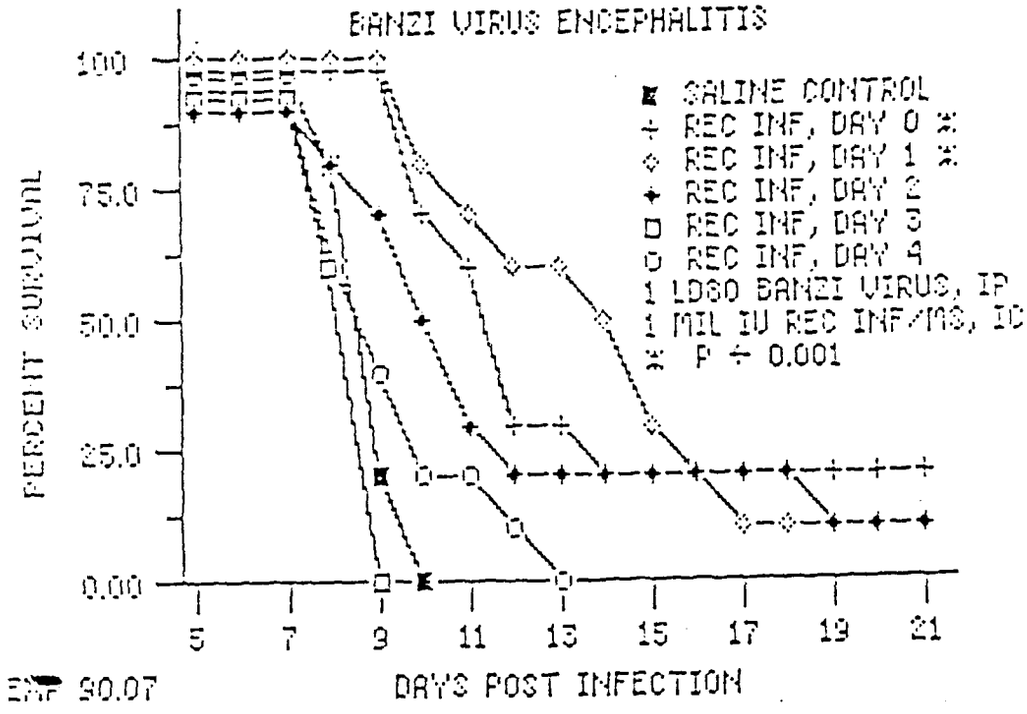
Mice were inoculated with a 100 μg/kg of CGP 31,362 on the days indicated. HSV-1 was administered intranasally to 4 week old male mice. Ten mice per treatment group were evaluated using Wilcoxon Rank Analysis.



Treatment	Geometric Mean Survival Time (days)	p Value
rhuIFN day		
0	17.8	<0.02
1	12.8	<0.05
2	9.5	NS
3	8.7	NS
Control (saline)	11.3	---

Figure 112. Effect of intravenously administered recombinant human interferon-α on Banzi virus-induced encephalitis.

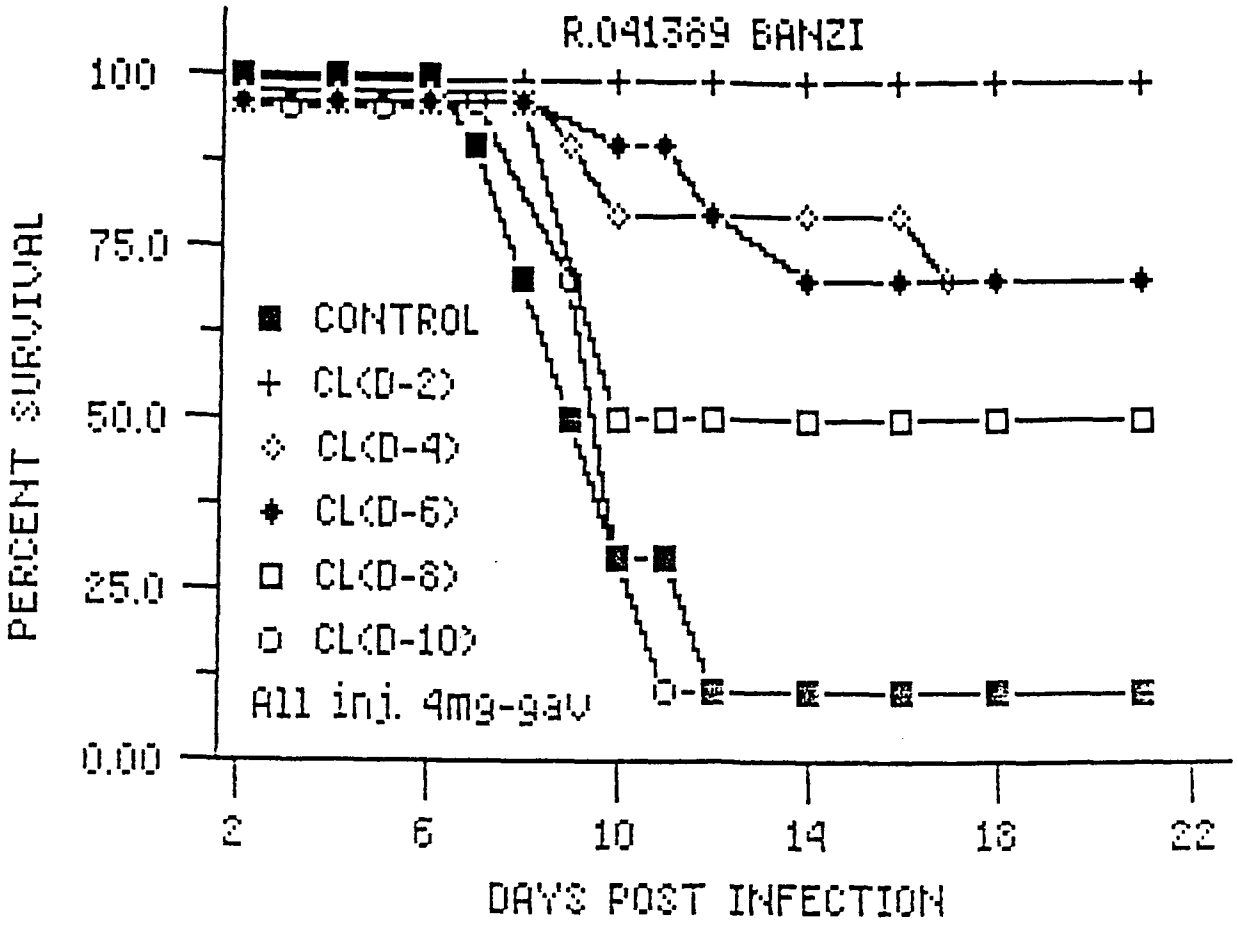
Mice were treated intravenously with rhuIFNα-B/D (1 x 10^6 IU) on the day indicated and challenged with 1 LD_{80} of Banzi virus subcutaneously. Ten mice per treatment group were analyzed using the Wilcoxon rank analysis.



Treatment	Geometric Mean Survival Time (Days)	p Value
rhuIFNα-B/D		
Day 0	11.4	<0.001
Day 1	13.3	<0.001
Day 2	10.8	NS
Day 3	8.6	NS
Day 4	9.5	NS
Saline Control	9.0	--

Figure 113. Intracranial administration of rhuIFNα-B/D in the therapy of Banzi encephalitis.

Mice were inoculated with a single dose (1 x 10^6 units) of interferon at the times indicated. Banzi virus (1 LD_{80}) was administered intraperitoneally to 6 week old male mice. Ten mice per treatment group were evaluated using Wilcoxon Rank Analysis.



Treatment	Geometric Mean Survival Time (Days)	p Value
CL 246738		
Day -2	21.0	<0.001
Day -4	17.5	<0.01
Day -6	17.7	<0.005
Day -8	14.5	NS
Day -10	10.6	NS
Saline Control	10.2	--

Figure 114. Prophylactic effects of CL 246738 in Banzi virus encephalitis.

Mice were given one dose of CL 246738 (200 mg/kg, orally) on the day indicated and challenged (subcutaneously) with 1 LD_{80} of Banzi virus. Ten mice per treatment group were examined using the Wilcoxon rank analysis.

PUBLICATIONS

Manuscripts

1. Gangemi, J.D., M. Nachtigal, D. Barnhart, L. Krech, and P. Jani. Therapeutic efficacy of liposome-encapsulated ribavirin and muramyl tripeptide in experimental infection with influenza or herpes simplex virus. J. Infect. Dis. 155, 510-517, 1989.

2. Kende, M., J.D. Gangemi, W. Lang, D.A. Eppstein, J. Kreuter, and P.G. Canonico. Carrier-mediated antiviral therapy. Appl. Virol. Res. 1, 241-264, 1988.

3. Gangemi, J.D., J. Lazdins, F.M. Dietrich, A. Matter, B. Poncionio, and H-K. Hochkeppel. Antiviral activity of a novel recombinant human interferon-α-B/D. J. Interferon Res. 9, 227-237, 1990.

4. Gangemi, J.D., A. Matter, B. Poncionio, and H-K. Hochkeppel. Significant differences in therapeutic responses to a human interferon-α-B/D hybrid in Rauscher or Friend murine leukemia virus infections. J. Interferon Res. 9, 275-283, 1990.

5. Lazdins, J. E. Alteri, K.W. Cook, C. Burgin and J.D. Gangemi. Use of human monocytes in the evaluation of antiviral drugs: Quantitation of HSV-1 cytopathic effects. Antiviral Research (In press).

6. Azmi, F.H., Ghaffar, A., Mayer, E.P., Mishell, R.T. and Gangemi, J.D. Immunomodulation by *Propionibacterium acnes*. II. Induction of cells that suppress anti-sheep erythrocyte antibody response. (Submitted).

Additional manuscripts comparing the efficacy of various immunomodulators in augmenting components of the immune system and in enhancing resistance to viruses are currently in preparation. These will be provided as they are completed.

Abstracts

1. Ghaffar, A., Mayer, E.P., Nachtigal, M. and Gangemi, J.D. Stimulation of nonspecific immune functions and enhancement of resistance to herpesvirus infections by Poly I:C-LC. Fed. Proc. 46:625 (abstr. 1800), 1987.

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